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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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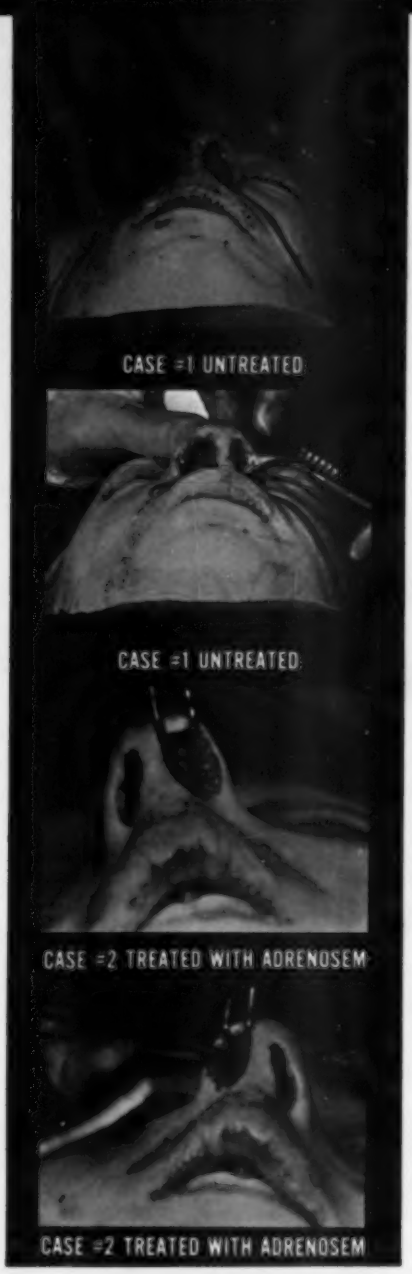

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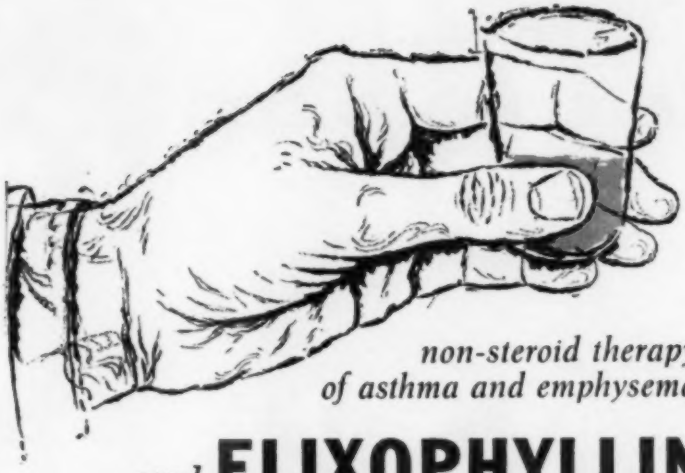

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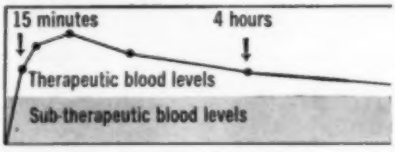

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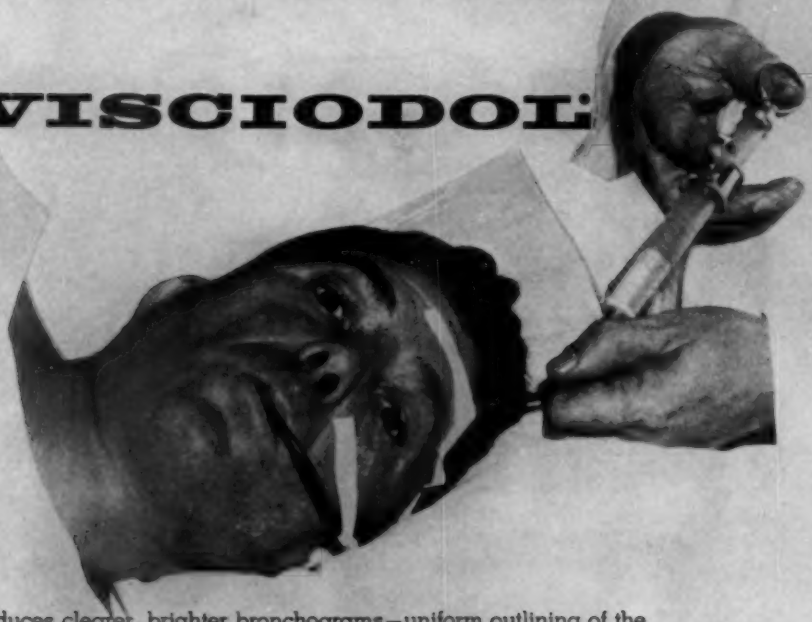

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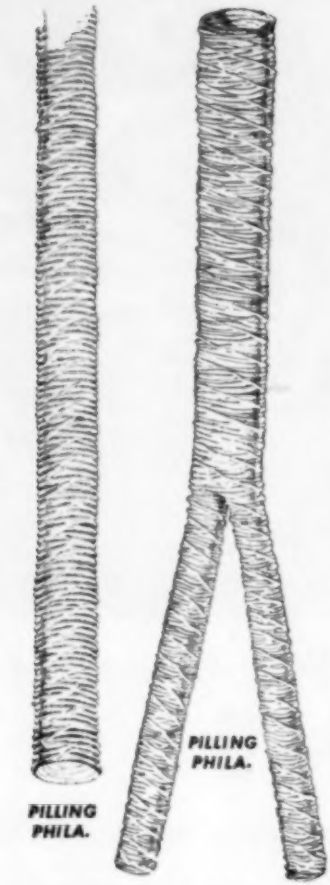

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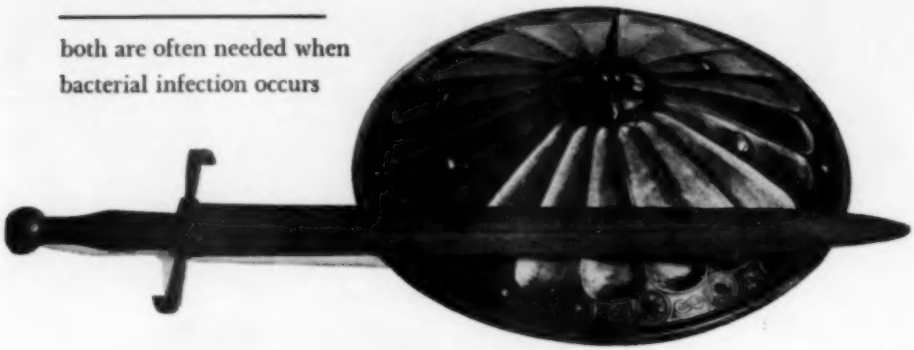

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ERRATUM

Subsequent to the publication of the article entitled "Lung Function in Silicosis of the Witwatersrand Gold Miner" by Becklake, DuPrez, and Lutz, in the March, 1958, issue of the REVIEW (vol. 77, p. 400), the authors noted that a few of the values in tables 1 and 4 were incorrectly presented and have supplied us with the corrected tables. These tables are reproduced below with the corrected values in italics:

TABLE 1

RESULTS OF LUNG VOLUME STUDIES, MIXING, AND EXERCISE VENTILATION

	Vital Capacity	Per Cent Predicted	Residual Volume	Per Cent Predicted	Functional Residual Capacity	Per Cent Predicted	RV / TLC × 100	FRC / TLC × 100	Mixing Index	Oximetry		Ventilation Equivalent for Oxygen		Hugh Jones Effort Test		
										Maximal Fall on Effort	Maximal Rise on Oxygen	At Rest	On Effort	Exercise Ventilation	Standard Ventilation	Dyspneic Index
Miners with no silicosis shown roentgenographically																
Aged 41–50	4.831	106.1	1.928	94.2	3.513	101.0	28.20	51.60	3.330	−1.34	6.30	4.35	2.99			
Standard deviation	0.753	15.0	0.507	25.4	0.737	20.4	*5.75*	8.97	0.558	1.77	1.38	1.42	0.56			
Aged 51–60	4.302	113.5	2.046	99.4	3.242	103.8	32.10	49.70	3.848	+0.02	5.56	4.57	3.16	33.90	35.30	30.90
Standard deviation	0.633	14.0	0.368	14.4	0.709	17.8	*4.23*	7.32	0.548	2.89	1.77	1.74	0.58	3.32	*3.67*	*7.86*
Miners with marked silicosis shown roentgenographically																
Aged 41–50	4.388	97.2	2.087	100.3	3.280	95.7	31.80	50.90	3.710	−2.07	6.13	5.77	3.51			
Standard deviation	0.850	15.7	0.572	23.6	0.779	15.0	*7.84*	8.09	0.645	2.08	2.18	1.94	0.72			
Aged 51–60	3.620	89.4	2.290	104.6	3.360	98.9	37.80	56.10	4.584	−1.04	4.56	5.24	3.39	35.95	37.55	54.00
Standard deviation	0.550	13.9	0.794	31.3	0.878	21.1	*8.75*	8.32	0.852	1.55	1.30	1.31	0.33	5.89	5.14	*20.40*

TABLE 4

PHYSICAL CHARACTERISTICS AND MINING SERVICE OF MINERS STUDIED

	Age	Height	Weight	Surface Area	Mining Service (years)	
					Total	Rock-breaking
	years	*cm.*	*kg.*	*sq. m.*		
Miners with no silicosis shown roentgenographically						
Aged 41–50	45.00	175.60	78.50	1.9360	17.00	4.60
Standard deviation	*2.21*	5.80	11.40	0.1637	3.07	5.77
Aged 51–60	54.80	169.00	74.40	1.8480	21.50	3.30
Standard deviation	*3.16*	4.59	7.62	0.1056	6.08	4.29
Miners with marked silicosis shown roentgenographically						
Aged 41–50	46.70	174.70	78.90	1.9380	17.90	9.50
Standard deviation	*3.40*	5.20	13.13	0.1454	4.72	4.19
Aged 51–60	54.70	173.60	76.90	1.9050	21.60	11.60
Standard deviation	*2.91*	4.58	8.11	0.1080	5.89	6.09

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 78 *November 1958* NUMBER 5

THE CHALLENGE OF TUBERCULOSIS TODAY

Keynote address of the Honorable John E. Fogarty, Member of the United States Congress from the Second District of Rhode Island, at the opening of the 1958 Annual Meeting of the National Tuberculosis Association, Philadelphia, Pennsylvania, May 19, 1958

It is most gratifying to be with you on this occasion. The annual meeting of the National Tuberculosis Association is, I know, a most significant occurrence in the nation's calendar of public health events.

In Washington where I serve, among other capacities, as chairman of the committee of the House of Representatives responsible for the appropriations of the Department of Health, Education, and Welfare, I am kept fully aware of your part in the progress that has been made in combatting tuberculosis. The story of your Association is a splendid record of achievement against great odds. Indeed, I believe that your efforts as community leaders and shapers of public opinion, and your vigorous cooperation with state, local, and federal health agencies, have been largely responsible for our present favorable position in the attack on a disease that has been a scourge from the beginning of recorded time.

There is another area of interest which you and I share. I refer to the field of medical research, including that aspect of medical research that has to do particularly with the prevention and treatment of tuberculosis.

For more than fifteen years I have derived great personal satisfaction from being privileged to have a part in the formulation of our national program for the conduct and support of medical research. I know the National Tuberculosis Association has made many notable contributions to research in its field of special interest—not only directly, through financial support of productive projects, but also indirectly, through calling the public's attention to research needs.

Through your rich relationships with the people—by means of your special knowledge, your insight, and perseverance—you build, in a real and vital sense, a portion of the health structure of tomorrow. The voluntary tuberculosis worker clearly perceives what must be done, day in and day out, to bring about the defeat of tuberculosis. He has the vision to see problems that require immediate action and to devise programs that will be uniquely suitable for his community to undertake.

Above all others, the voluntary health worker, because of his closeness to the people, is most keenly aware of the feasibility and practicality of plans for public health action. No one from Washington or anywhere else can tell him what the people are ready for and what methods he must use to achieve his goals.

Indeed, the pattern of future action in the control of tuberculosis, as well as the course of *total* public health activity, depends in large part on the understanding, the community acceptance, and the foresight of the local voluntary health worker. His effectiveness, in turn, is determined by the degree of his identification with the people of his community.

As one who is privileged to serve in the House of Representatives, I have frequently had occasion to witness, to participate in, and to reflect on the fundamental meaning of the democratic process, especially as it applies to the health and welfare of the American people.

Throughout the hearings on federal health and welfare appropriations over which I preside, I am always impressed by the deep humane concern of the men and women who serve the people's purposes through the instrumentalities of government. Repeatedly, it is made clear to me that, if there is one characteristic of American life that sets us apart from other nations, it is the way in which our people give expression to their will and desire.

As a result of concerted citizen action, we have achieved in our nation a proud record in public health and welfare.

This annual meeting is an excellent example of American democratic action. Without such an organization as yours, present achievements in the control of tuberculosis could not have been realized. Our strong federal, state, and local official agencies could not have come into being and could not have grown to their present stature without the united forces of citizen opinion and action. Our American willingness to cooperate toward the achievement of desirable goals, our people's generosity with money, time, and energy have enabled us to succeed in the control of a devastating disease like tuberculosis as they have permitted us to prosper as a nation.

My work in Congress and my continuing concern for and interest in public health problems permit me to believe that this morning I am among allies. There is, therefore, no need for me to entreat you, to recruit, or to justify. As citizens, we are in the same struggle. We work toward the same end—the defeat of tuberculosis. And though you are experts and I am not, we speak essentially the same language, one that bespeaks our desire to be free from a lethal enemy of mankind.

I do not have to convince you of the seriousness of tuberculosis, nor do I need to describe the almost miraculous advances that have been made toward its conquest. Progress in solving the problems of tuberculosis is something that we sense, even in the absence of statistical proof, as a reality. We do not need constant reassurance to know that we are steadily approaching the ultimate goal—the elimination of tuberculosis as a public health problem. However, each step closer to that objective exposes new and challenging problems which demand solution. And if you, as leaders in a disease-control movement that reaches every home in your states, are to reach that goal in the foreseeable future, you must be convinced of the still urgent nature of your mission. You must continue to demonstrate to the people the real need for continuing, unrelenting effort.

To be sure, it is deeply satisfying to live and work in an era when, for the first

time in countless centuries, tuberculosis can be—and in this country has been—reduced to virtual impotence as a dealer of death. But there is danger in such satisfaction, for it may weaken our awareness of the inescapable fact that tuberculosis still remains a mighty threat as a source of prolonged illness, disrupted families, personal anguish, and lost economic productiveness.

As a layman who tries to keep informed in health matters and as a Representative who is deeply concerned about organized programs in the prevention and control of tuberculosis, I am greatly encouraged, as you are, by the continuing decline of the death and case rates. The Public Health Service informs me that since 1939 the death rate has declined in the United States from 47.1 per 100,000 population to 8.3 in 1956. Provisional figures show a further decline for 1957.

Indeed, with every passing year fewer people die of tuberculosis; illness from the disease decreases in frequency and severity; and fewer children and young adults are infected. The year 1956 is a milestone in the history of the attack on tuberculosis. In that year newly reported cases showed a decline more precipitous than any in recent years. For the first time morbidity exhibited a greater decline than mortality. Newly reported cases fell 10 per cent below the figures for 1955; the death rate fell by 6 per cent. It cannot be said yet that this is a real breakthrough; but it does indicate a leveling off of the death rate, while reported cases continue downward.

Nevertheless, nearly 14,000 persons died last year from tuberculosis. Sixty-nine thousand new, active, and probably active cases were reported to health authorities. There are 250,000 active cases of tuberculosis in the United States today plus 550,000 inactive cases that require public health supervision and service. These active and inactive cases, old cases, and the millions of infected people constitute a reservoir of disease that could reverse the great advances of the past.

Progress always presents new problems. At this very moment there are approximately 300,000 tuberculous persons in this country who are not in hospitals and who are in need of supervision and service. In most of these cases, drugs must be administered and clinical services provided over a period of eighteen to twenty-four months after hospital discharge. However, studies conducted by the Public Health Service show that a startling proportion of these patients are not receiving adequate care and supervision. For the country as a whole, there has not yet been developed a workable system whereby persons with tuberculosis outside hospitals can realize the full benefits of modern medical and public health resources. Now, and in the future, much of this responsibility for providing and arranging the services needed by tuberculosis patients outside hospitals will rest on state and local health departments.

However, if this disease is to be kept under control and the affected individuals restored to productive lives, these patients, the potential sources of new disease, need drugs, continuing medical and nursing supervision, and essential social and economic counsel. Only in this way will the public as a whole, as well as these sick persons, receive the full benefits of the great therapeutic gains of the recent past.

I have left to the last a discussion of research, because I wish to place special emphasis on it as a predominant factor in the future of the tuberculosis control movement.

Not many years ago it was not uncommon for authorities in the field of tuberculosis to express the conviction that sufficient knowledge was already available to make the eradication of tuberculosis a possibility within a few generations if established control techniques were effectively applied. Moreover, interest in research was, until very recent years, limited to laboratory investigations, and responsibility for it was largely centered in special institutions and groups. But today we see new support for research in tuberculosis and new evidence of widespread interest in epidemiologic as well as laboratory studies. Moreover, responsibility for research has been accepted by a wide range of operating programs as well as by special institutions. A most significant development, indeed, has been the growth of cooperative clinical investigations which make use of multiple disciplines, staffs, and facilities, thereby permitting nation-wide research studies that include large and diverse population samples.

I should like to cite two examples of such research now being carried on by the Public Health Service or through projects supported by the National Institutes of Health. They are: (*1*) the careful testing, by means of controlled studies, of the effectiveness of isoniazid as a preventive, or prophylactic, of tuberculosis; (*2*) the development of a vaccine that does not have the disadvantages of BCG.

Studies of isoniazid as a prophylactic, conducted by the Public Health Service in cooperation with the National Tuberculosis Association, showed such promise among laboratory animals that trials of the drug as a preventive of tuberculosis in human beings have been organized and are now going forward in numerous communities throughout the country.

An initial project, involving more than 2,700 children in 33 pediatric clinics in the United States, Puerto Rico, Mexico, and Canada, tested the effect of isoniazid in preventing complications of primary tuberculosis. The first results from this work show that, during the twelve months in which these children have been taking isoniazid, at least 80 per cent of the major complications of childhood tuberculosis were prevented. On the basis of these findings, health agencies and private physicians are provided with a new, well-confirmed method of control that can be applied now.

To complement this knowledge, further research is required to determine whether this protection continues for an extended period after the drug is discontinued and whether similar protection can be given other population groups under different conditions. Therefore, studies among close contacts of newly discovered cases of tuberculosis are being carried out. Trials have already begun in about 40 communities and institutions in 16 states. Additional groups are being added to this research project monthly. A peak workload on this research will probably be reached in fiscal year 1959.

The second aspect of research, the development of a vaccine, has become a subject of increasing interest to me. The experts tell me that we need a vaccine which may be given to everybody regardless of his reaction to the tuberculin

test; one which is, of course, not only safe but without reactions of any consequence; one preferably consisting of killed microorganisms; and one which produces a solid immunity. If and when such a vaccine is available, mass immunization of the entire population may have top priority in the tuberculosis control program.

Research now going on under the auspices of the National Institutes of Health and elsewhere gives some promise that such a vaccine may ultimately be realized. But, of course, it is too early now to make predictions. The development of a vaccine, as you well know, is a long, laborious, arduous task. You may be assured, however, that this work will have my abiding interest and persistent support. Perhaps, in some future year not too far distant, you may again invite me to attend your annual meeting, and I may then be able to discuss with you a great achievement in the field of tuberculosis vaccination. Let us hope.

* * *

There are many other areas of action and fact finding that might well engage our attention this morning, but their proper forum is in your discussion sessions and scientific meetings. I want merely at this time to salute you for past work ably done and to express my deeply felt wishes for the future.

Despite the magnitude of your achievements, the job that remains to be done is enormous.

It is easy to formulate a hundred unanswered questions in the field of tuberculosis control, those of poverty, malnutrition, mental and emotional imbalances, unhealthful and crowded housing, industrial health hazards—all of these plead for your attention and action insofar as they contribute to continuing illness from tuberculosis.

Although tuberculosis has diminished in force as a *lethal* enemy of mankind, it will remain a threat as long as the tubercle bacillus continues to infest the human body. The history of tuberculosis demonstrates again and again the insidious resourcefulness of this bacillary organism.

We all know that the mortality rate alone does not reflect the social menace of tuberculosis. Indeed, it is through prolonged destructive illness that tuberculosis takes its social and economic toll. To be sure, it is a task of prime magnitude to save people from untimely death. But the way to prevent these deaths is to fight tuberculous *disease*. The incapacitating character of tuberculosis, the broken homes, the loss of productivity constitute the essential challenge. Even were there no deaths from tuberculosis, its devastation as a long-term illness would demand our utmost vigilance.

I know that you will go on to even greater achievements if you are careful not to cling to these that are finished. We all must be willing to accept changes and face new challenges with unremitting vigor and unflagging imagination. Moreover, you must never lose sight of the irrefutable fact that the success of the voluntary movement in tuberculosis control depends upon a broad base of citizen cooperation, support, and action.

You must fight complacency on all sides and refute the smug assumption

that in tuberculosis little remains to be done. No thinking person can be complacent in the face of the long list of unfinished business, not only among tasks not yet undertaken but also among tasks not yet done well enough.

Yes, there is still much to do. Citizens, now as in the past, will tackle new challenges with the resolute vigor that has always characterized American democratic action. But let us remember, however, that all our future public health work depends not only upon organizational functioning but also upon the kind of public interest and understanding that tuberculosis associations have persistently stimulated and maintained.

AN EVALUATION OF TUBERCULOSIS CASE FINDING BY TUBERCULIN TESTING AND SOME OBSERVATIONS OF HISTOPLASMIN SENSITIVITY AMONG YOUNG SCHOOL CHILDREN[1,2]

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INTRODUCTION

Yearly tuberculin and histoplasmin skin tests among children of the kindergarten and first grades of the public schools in Kansas City, Missouri, have been performed for the past ten years. The present study was directed primarily toward tuberculosis but, since histoplasmosis often presents a problem of differential diagnoses in this area, simultaneous histoplasmin and tuberculin tests were applied. A preliminary report of this study has previously been presented (1, 2).

The purpose of the present report is to summarize the results of the ten-year study with particular reference to the annual prevalence rates for tuberculin and histoplasmin sensitivity, conversion rates for these antigens, and to the yield of tuberculosis among the tuberculin reactors as well as among their household and nonhousehold contacts. Further analysis of the results will show the correlation of the size of the tuberculin reaction with the yield of tuberculosis. Estimates of the cost of finding tuberculosis by this method are also presented.

MATERIALS AND METHODS

Tuberculin and histoplasmin skin tests were performed annually for the years 1947-1957. During the last seven years of this period, nonreactors in the kindergarten group were re-tested the following year. A child whose skin test was negative one year and positive the next year was classified as a converter. All reactors to either antigen received a 70 mm. roentgenogram and, if any abnormality was noted, a standard-sized roentgenogram was made.

The household and nonhousehold contacts of all tuberculin-positive children and the "converters" to the tuberculin test were interviewed and urged to accept the same roentgenographic procedure as outlined for the index children. If any abnormality was noted on the standard-sized film of either child or adult, they were referred to their family physician or the Health Department Tuberculosis Clinic for clinical evaluation. If this evaluation established a diagnosis of tuberculosis, the case was classified as "new" or "known," based on the Health Department Tuberculosis Register. No follow-up program among contacts of histoplasmin reactors was conducted.

The tuberculin employed in this study was purified protein derivative (PPD). During the first seven years of this survey, commercial PPD was used, and during the last three years the international standard PPD-S as supplied by the U. S. Public Health Service was used. The dose was 0.0001 mg. or 5 tuberculin units. The histoplasmin used was Lot H 42 in a 1:100 dilution, as supplied by Dr. C. E. Palmer of the U. S. Public Health Service

[1] From the Heart of America Tuberculosis Association, Kansas City, Missouri; the University of Kansas School of Medicine, Kansas City, Kansas; and the Communicable Disease Center, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

All tests were administered intracutaneously by a physician and a nurse working as a team. Steel needles were used for seven years, and no complications were encountered. However, during the past three years platinum needles have been used and the needle was flamed after each test was given. One drop of the test solution was then expelled through the needle and discarded to eliminate any coagulum.

The tests were read by a physician at forty-eight hours. The total transverse measurement in millimeters of erythema and induration was recorded. A positive reaction was one in which the induration measured 5 mm. or more.

For the past five years each school was required to obtain parental consent for at least 75 per cent of the children to participate before the tests were given. However, there was always considerable loss due to absenteeism. Based on the number of tests read, an average of 69.3 per cent of the eligible school population participated.

During the ten-year study, a total of 66,033 tests were performed on 51,040 children. There were 35,995 children tested for the first time in the kindergarten, and 15,045 children received their first test while in the first grade and naturally would not be re-tested. In the seven years of re-testing of the nonreactors, 14,993 children whose skin tests were negative in the kindergarten were re-tested in the first grade the following year.

Results

The results of these studies substantiate those previously reported on the prevalence of tuberculin and histoplasmin sensitivity among kindergarten and first-grade children in the public schools of Kansas City, Missouri (1, 2).

The prevalence of tuberculin reactors among 35,995 kindergarten children observed in the ten years between 1947–1957 is shown in table 1. In this table also may be seen a comparison of the prevalence rates for white and Negro children. Although there is some variation from year to year, the over-all tuberculin sensitivity rate among Negroes is almost twice that of whites (2.6 per cent versus 1.5 per cent).

TABLE 1

Percentage of Tuberculin Reactors Among 29,632 White and 6,363 Negro Kindergarten Children in Kansas City, Missouri 1947–1957

School Year	Percentage Tuberculin Positive					
	White			Negro		
	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive
1947–1948	2,505	17	0.7	473	11	2.3
1948–1949	2,616	67	2.6	391	15	3.8
1949–1950	2,666	47	1.7	467	8	1.7
1950–1951	2,524	32	1.3	439	13	3.0
1951–1952	2,915	47	1.6	449	13	2.9
1952–1953	3,267	39	1.2	681	16	2.3
1953–1954	3,469	51	1.5	740	26	3.5
1954–1955	3,144	36	1.1	811	24	3.0
1955–1956	3,234	34	1.1	892	21	2.4
1956–1957	3,292	60	1.8	1,020	20	2.0
Totals, 1947–1957	29,632	430	1.5	6,363	167	2.6

TABLE 2

PERCENTAGE OF HISTOPLASMIN REACTORS AMONG 29,634 WHITE AND 6,369 NEGRO KINDERGARTEN CHILDREN IN KANSAS CITY, MISSOURI 1947–1957

School Year	Percentage Histoplasmin Positive					
	White			Negro		
	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive
1947–1948	2,494	322	12.9	473	26	5.5
1948–1949	2,580	294	11.4	391	34	8.7
1949–1950	2,669	333	12.5	455	40	8.8
1950–1951	2,536	299	11.8	439	29	6.6
1951–1952	2,927	319	10.9	457	45	9.8
1952–1953	3,273	348	10.6	680	63	9.3
1953–1954	3,474	316	9.1	742	63	8.5
1954–1955	3,146	240	7.9	811	76	9.4
1955–1956	3,239	286	8.8	895	73	8.2
1956–1957	3,296	337	10.2	1,026	104	10.1
Totals, 1947–1957	29,634	3,094	10.4	6,369	553	8.7

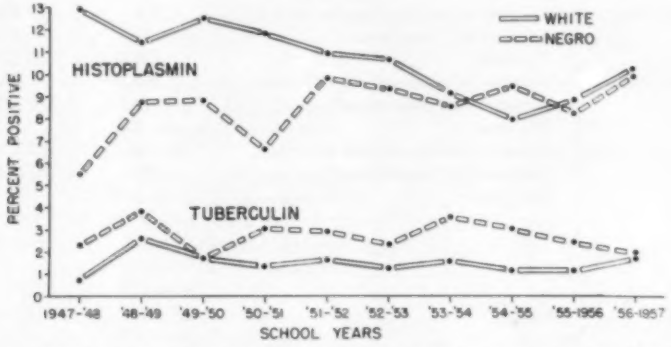

FIG. 1. Percentage of tuberculin and histoplasmin reactors among 29,632 white and 6,363 Negro kindergarten children in Kansas City, Missouri, 1947–1957.

The prevalence of histoplasmin reactors among the kindergarten children is shown in table 2. In contrast to the rates shown for tuberculin sensitivity, the histoplasmin rates for the entire group are higher for white children than for Negroes (10.4 per cent versus 8.7 per cent).

The combined data from tables 1 and 2 are shown in figure 1. This presents a comparison of prevalence rates for histoplasmin and tuberculin reactors for both white and Negro children. The tuberculin sensitivity rates were shown to be remarkably consistent from year to year and showed no tendency to decline over the entire ten-year period of observation.

TABLE 3

"CONVERTERS" TO TUBERCULIN TEST

NUMBER AND PERCENTAGE OF CHILDREN WHOSE TUBERCULIN TESTS WERE NEGATIVE IN KINDERGARTEN AND POSITIVE IN THE FIRST GRADE ONE YEAR LATER

Seven Years of Observation

School Year		White	Negro
1950–1951	Number of nonreactors re-tested	1,417	270
	Number of "converters"	11	6
	Per cent	0.8	2.2
1951–1952	Number of nonreactors re-tested	1,372	255
	Number of "converters"	15	3
	Per cent	1.1	1.2
1952–1953	Number of nonreactors re-tested	1,560	260
	Number of "converters"	14	5
	Per cent	0.9	1.9
1953–1954	Number of nonreactors re-tested	2,002	455
	Number of "converters"	14	5
	Per cent	0.7	1.1
1954–1955	Number of nonreactors re-tested	1,963	450
	Number of "converters"	24	4
	Per cent	1.2	0.9
1955–1956	Number of nonreactors re-tested	1,879	495
	Number of "converters"	17	8
	Per cent	0.9	1.6
1956–1957	Number of nonreactors re-tested	2,060	555
	Number of "converters"	22	7
	Per cent	1.1	1.3
Combined years	Number of nonreactors re-tested	12,253	2,740
	Number of "converters"	117	38
	Per cent	1.0	1.4

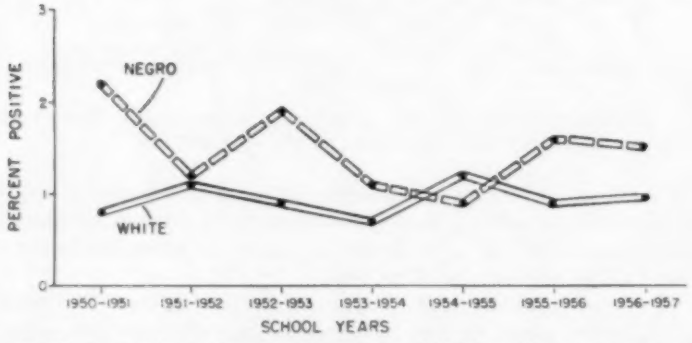

FIG. 2. "Converters" to tuberculin. Percentage of children whose tests were negative in kindergarten and positive in the first grade one year later: 12,253 white and 2,740 Negro children; seven years' observation.

The average of the histoplasmin rates over the same period of years is higher for white children than for Negroes. The graph shows a rather wide difference in the rates at the beginning of this study for 1947–1948. However, subsequent annual rates for white children showed a tendency to decrease while those for Negro children increased so that for the final two years of observation they were almost the same. The epidemiologic factors involved in this change are not known.

The number and percentage of children whose tuberculin tests were negative in kindergarten and positive in the first grade one year later are shown in table 3. As previously shown in the prevalence of tuberculin reactors (table 1), the percentage of tuberculin converters is higher among Negroes than among whites, the average over the seven-year period of observation being 1.4 and 1.0 per cent, respectively.

A comparison of the percentage of tuberculin converters among the 12,253 white and 2,740 Negro children is shown in figure 2. As with the prevalence of

TABLE 4

"CONVERTERS" TO HISTOPLASMIN TEST

NUMBER AND PERCENTAGE OF CHILDREN WHOSE HISTOPLASMIN TESTS WERE NEGATIVE IN KINDERGARTEN AND POSITIVE IN THE FIRST GRADE ONE YEAR LATER

Seven Years of Observation

School Year		White	Negro
1950–1951	Number of nonreactors re-tested	1,261	258
	Number of "converters"	45	13
	Per cent	3.6	5.0
1951–1952	Number of nonreactors re-tested	1,256	230
	Number of "converters"	21	16
	Per cent	1.7	7.0
1952–1953	Number of nonreactors re-tested	1,387	246
	Number of "converters"	40	11
	Per cent	2.9	4.5
1953–1954	Number of nonreactors re-tested	1,843	426
	Number of "converters"	34	18
	Per cent	1.8	4.2
1954–1955	Number of nonreactors re-tested	1,822	440
	Number of "converters"	59	18
	Per cent	3.2	4.1
1955–1956	Number of nonreactors re-tested	1,761	458
	Number of "converters"	38	15
	Per cent	2.2	3.3
1956–1957	Number of nonreactors re-tested	1,913	516
	Number of "converters"	55	32
	Per cent	2.9	6.2
Combined years	Number of nonreactors re-tested	11,243	2,614
	Number of "converters"	292	123
	Per cent	2.6	4.7

reactors, there was no tendency for the tuberculin conversion rates to decline during this period of observation.

The number and percentage of white and Negro children whose histoplasmin tests were negative in kindergarten and positive in the first grade one year later are shown in table 4. It has been noted (table 2, figure 1) that during the first few years of this study the histoplasmin prevalence rates among white children were higher than among Negroes, but during the last years were approximately the same. The annual histoplasmin conversion rates, however, were consistently higher among the Negro children, the average for Negro children being 4.7 per cent and for white children, 2.6 per cent.

A graphic comparison of these histoplasmin conversion rates among 11,243 white and 2,614 Negro children is shown in figure 3.

The close relationship between tuberculin sensitivity rates and tuberculosis mortality rates for the white population is shown in table 5. There was considerable variation in the tuberculin sensitivity rate by section of the city. The highest percentage of reactors was found in the northwest section. This is the oldest and most densely populated area of the city and is occupied principally

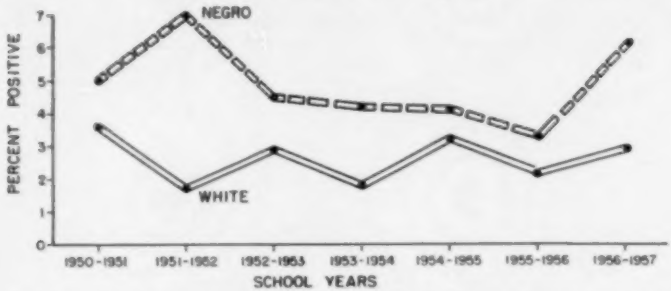

FIG. 3. "Converters" to histoplasmin. Percentage of children whose tests were negative in kindergarten and positive in the first grade one year later: 11,243 white and 2,614 Negro children; seven years' observation.

TABLE 5

COMPARISON OF TUBERCULIN SENSITIVITY WITH TUBERCULOSIS ANNUAL DEATH RATES BY SECTIONS OF KANSAS CITY, MISSOURI

1947–1957

White Only

	Sections of the City				
	NE	NC	NW	SE	SW
Tuberculin sensitivity in kindergarten (public schools), 1947–1957	1.2%	1.6%	2.8%	1.3%	1.1%
Tuberculosis annual death rate per 100,000,* 1949–1956	9.8	20.6	45.9	8.4	7.0

* Based on populations as determined by 1950 census.

by persons with relatively low incomes. It will be noted that the section of the city having the highest tuberculin sensitivity rate also had the highest mortality rate, and the section having the lowest sensitivity rate also had the lowest mortality rate.

A comparison of induration and erythema measurements among 29,202 white kindergarten children who were classified as nonreactors to tuberculin is shown

TABLE 6

COMPARISON OF INDURATION AND ERYTHEMA MEASUREMENTS AMONG 29,202 WHITE KINDERGARTEN CHILDREN CLASSIFIED AS NONREACTORS TO TUBERCULIN

1947–1957

Erythema	Induration (mm)				Total
	0	2	3	4	
mm.					
0	28,350				28,350
2	72	114			186
3	84	38	50		172
4	73	43	36	6	158
5	60	37	38	32	167
6	12	4	18	12	46
7	14	3	8	10	35
8	10	4	10	2	26
9	6	3	3	7	19
10	10	5	2	3	20
11	3		1	3	7
12	5			1	6
13 and more	3	1	4	2	10
Total	28,702	252	170	78	29,202

TABLE 7

FREQUENCY DISTRIBUTION OF REACTION SIZES FOR CHILDREN POSITIVE ON THE FIRST TEST, KINDERGARTEN, KANSAS CITY, MISSOURI

1947–1957

Size of Reaction	Total Positive	
	Number	Per Cent
mm.		
5–9	260	61
10–14	69	16
15–19	45	10
20–24	31	7
25 and more	17	4
Unknown	8	2
Total	430	100

in table 6. Among this group of children there were 500 who showed some readable induration and erythema at the site of injection less than the acceptable standard for a positive reaction. There were also 352 children whose tests produced only erythema. Comparison of the erythema and induration measurements among the group of white children whose reactions were considered positive (induration 5 mm. or more) revealed that the measurement of the erythema averaged 8 mm. more than that of the induration. The frequency distribution of reaction sizes for white kindergarten children positive on the first test is shown in table 7.

Results of Follow-up Studies

The follow-up studies as previously outlined were conducted on a current school-year basis. A few cases of tuberculosis among contacts developed subsequent to the initial examination, but these were not included unless the discovery of the disease resulted from the follow-up study of another index child.

The yield of tuberculosis among the contacts of 155 tuberculin converters and of 833 children who were reactors on the first test is shown in table 8. The results include adults and children and both white and Negro household and nonhousehold contacts. These data show that an average of approximately six contacts for each converter and four and one-half for each child who was a tuberculin reactor on the first test were followed. The average yield of new active previously unknown tuberculosis (1.2 per cent) was the same for the converters as for those who were reactors on the first test. The yield of known tuberculosis among contacts of children who were tuberculin reactors at the time of entrance to kindergarten was almost twice that of the converters (4.1 per cent versus 2.3 per cent). This might be expected since the potential time of exposure to infection is five years for one group and one year for the other.

TABLE 8

FOLLOW-UP STUDY OF TUBERCULIN REACTORS

COMPARISON BETWEEN CONVERTERS AND CHILDREN POSITIVE ON THE FIRST TEST: RESULTS INCLUDE BOTH WHITE AND NEGRO HOUSEHOLD AND NONHOUSEHOLD CONTACTS

1947–1957

Tuberculin Status of Index Child	Household and Nonhousehold Contacts	Active Tuberculosis					
		New		Known		Total	
		Number	Per Cent	Number	Per Cent	Number	Per Cent
155 converters	655 adults	7	1.1	18	2.7	25	3.8
	273 children	4	1.5	3	1.1	7	2.6
	Total 928	11	1.2	21	2.3	32	3.4
833 positive on first test	2,602 adults	32	1.2	147	5.6	179	6.9
	1,133 children	13	1.1	7	0.6	20	1.8
	Total 3,735	45	1.2	154	4.1	199	5.3
Grand total	4,663 adults and children	56	1.2	175	3.6	231	5.0

TABLE 9

FOLLOW-UP STUDY OF TUBERCULIN REACTORS

COMPARISON OF WHITE AND NEGRO CHILDREN: RESULTS INCLUDE CONVERTERS AND CHILDREN POSITIVE ON FIRST TEST AND WHITE AND NEGRO HOUSEHOLD AND NONHOUSEHOLD CONTACTS

1947–1957

	Household and Nonhousehold Contacts	Active Tuberculosis					
		New		Known		Total	
		Number	Per Cent	Number	Per Cent	Number	Per Cent
766 white children	2,703 adults	30	1.1	118	4.4	148	5.5
	1,122 children	13	1.2	7	0.6	20	1.8
	Total 3,825	43	1.1	125	3.3	168	4.4
222 Negro children	554 adults	9	1.6	47	8.5	56	10.1
	284 children	4	1.4	3	1.1	7	2.5
	Total 838	13	1.6	50	6.0	63	7.5

TABLE 10

FOLLOW-UP STUDY OF TUBERCULIN REACTORS

COMPARISON OF HOUSEHOLD AND NONHOUSEHOLD CONTACTS: RESULTS INCLUDE BOTH WHITE AND NEGRO HOUSEHOLD AND NONHOUSEHOLD CONTACTS

1947–1957

		Active Tuberculosis					
		New		Known		Total	
		Number	Per Cent	Number	Per Cent	Number	Per Cent
2,908 household contacts	1,859 adults	25	1.3	69	3.7	94	5.1
	1,049 children	17	1.6	9	0.9	26	2.5
	Total 2,908	42	1.4	78	2.7	120	4.1
1,755 nonhousehold contacts	1,398 adults	14	1.0	94	6.7	108	7.7
	357 children	0	0.0	1	0.3	1	0.3
	Total 1,755	14	0.8	95	5.4	109	6.2

A comparison of the tuberculosis found among the contacts of white and Negro children is shown in table 9. In this table the tuberculin converters and children positive to the first test are combined. It is evident that, while the yield of new active tuberculosis is a little higher among contacts of Negro children than among those of white children, the follow-up results were essentially the same in the two groups. However, the yield of known tuberculosis among the Negroes was almost twice that of the yield in whites.

The results of follow-up study of the household and nonhousehold contacts of all tuberculin reactors are shown in table 10. This shows that the yield of new previously unknown tuberculosis is almost twice as high among household contacts as among nonhousehold contacts (1.4 per cent versus 0.8 per cent). How-

TABLE 11

FOLLOW-UP STUDY OF CONTACTS OF CONVERTERS AND CHILDREN POSITIVE ON THE FIRST TEST BY REACTION SIZES OF INDEX CHILD, WHITE ONLY 1947–1957

Size of Reaction of Index Child	Number of Children	Number of Household and Nonhousehold Contacts	Active Tuberculosis					
			New		Known		Total	
			Number	Per Cent	Number	Per Cent	Number	Per Cent
mm.								
5–9	409	2,214	6	0.3	48	2.2	54	2.4
10–14	139	691	12	1.7	28	4.1	40	5.8
15–19	68	333	5	1.5	17	5.1	22	6.6
20–24	45	238	5	2.1	17	7.1	22	9.2
25 and more	27	113	10	8.8	6	5.3	16	14.2

ever, known tuberculosis was found to be much more prevalent among nonhousehold contacts (5.4 per cent) than among the household contacts (2.7 per cent).

One of the most interesting findings of this study is the relationship of tuberculosis found among contacts and the size of the tuberculin reaction of the index child, as shown in table 11. Although the total number of persons involved is relatively small for this type of study, the findings seem significant. The yield of tuberculosis among contacts (in per cent) rises steadily as the size of the reaction of the index child increases. Only 0.3 per cent of the contacts of minimal reactors (5 to 9 mm.) were found to have new active tuberculosis as compared to 8.8 per cent of those in contact with the most intense reactors (25 mm. or more). It is evident that in this study approximately thirty times as much new tuberculosis was found among the contacts of children whose tuberculin reaction exceeded 25 mm. induration as among those whose reaction was 5 to 9 mm.

Cost of Skin Test Follow-up Studies

Attempts were made to approximate the cost of the program. This estimate was based on the expense of one school year, 1955–1956. A physician was employed to make all readings and to administer one of the tests, while the other was administered by a nurse. The cost of such a program, including follow-up observation of tuberculin-positive children and their contacts, but without the cost of the roentgenogram, was about 68¢ per child. When roentgenograms of the tuberculin-positive children and contacts were added to the above program, the cost rose to 81¢ per child. When the complete program was carried out, including two skin tests, roentgenograms of tuberculin- and histoplasmin-positive reactors and of the contacts of the tuberculin reactors, the cost rose to $1.03 per child.

The cost of this program may seem relatively high; nevertheless, when costs relating only to follow-up study of tuberculin-positive children and contacts are considered, the cost of finding a new active case of tuberculosis was approximately $823.00. This figure compares favorably with the cost estimate for finding a new

case by the mass roentgenography program. To find any case of tuberculosis, new or known, the cost was approximately $201.00.

Discussion

Recently there has been renewed interest in the tuberculin test as a method of tuberculosis case finding. This interest has been stimulated largely by three factors. First, mass roentgenographic surveys become less efficient and consequently more expensive as the tuberculosis case rate diminishes. Furthermore, participation of the eligible age groups is often disappointing, particularly when re-survey of the same group is attempted. Second, the efficiency of the tuberculin test as a case-finding procedure increases as the general prevalence of tuberculin sensitivity decreases. Recently, a third factor of minimizing the exposure to ionizing radiation has been introduced. This study included roentgenograms of tuberculin reactors and their close contacts only and thus represents a fairly high degree of selective roentgenographic exposure.

A proper evaluation of any tuberculosis case-finding method must be based on certain fundamentals which include the ease of performance, the cost, and the number of cases of active tuberculosis found.

The youngest of school children were selected for this study because, if tuberculous infection had occurred, it would be relatively recent and their contacts would be limited and easier to follow. The yield of new tuberculosis cases among the children was low, as would be expected, but among the adults there was a surprising number of new previously unknown cases of tuberculosis; the average being about twelve times that obtained by mass roentgenographic survey. A high percentage of active tuberculosis was found among the previously known cases. Most of these persons had received some form of treatment. A definite analysis of the previously known cases of tuberculosis was not within the scope of this study. However, information indicates that the disease was considered inactive in many of these cases and that further follow-up examination had been discontinued either by the patient, his family physician, or the clinic physician. This again emphasizes the importance of continued close observation of all known tuberculous patients. This is particularly urgent in view of the fact that an accelerated hospital treatment program or ambulatory treatment has been accepted in many communities.

The method of referral of all persons with suspicious roentgenographic findings to their private physicians or the Tuberculosis Clinic for evaluation may be open to criticism. Certainly, information of an accurate diagnosis was difficult to obtain in some cases. However, there were certain collateral benefits which, in the opinion of the present writers, compensated for this shortcoming. They believe that they have fostered a tuberculosis educational program among physicians by having a well-informed public health nurse call at the physician's office and present him with the facts already obtained concerning his patient and discuss the procedure by which these facts were obtained. It is then his responsibility to perform further definitive diagnostic tests and, if active tuberculosis is found, to arrange to break the family contact, preferably by hospitalizing the patient. The

maintenance of this doctor-patient relationship has afforded good rapport and cooperation with both the patient and his family physician.

Another of the intangible benefits of this survey is the educational program conducted among school administrators, nurses, and parent-teacher organizations prior to testing and by interviews with the parents of tuberculin reactors. It is believed by the writers that the personal interviews with the parents of an index child and with their physician are effective in promoting tuberculosis control.

This survey revealed another fact of considerable public health interest. In this community the percentage of children infected with tubercle bacilli at the time of entrance into school is the same now as it was ten years ago. Furthermore, the percentage of children who became infected during their first year in school was relatively high and showed no tendency to decline over the period of seven years' observation. These relatively constant annual tuberculin prevalence and conversion rates should represent a reasonably accurate index of tuberculosis control within the city. There appears to be no ready explanation of the failure of these rates to fall when the death rate for Kansas City has fallen from 31.3 to 11.7 during the ten years of the survey. Reliable morbidity rates are not available. There are other possible explanations for the unchanged prevalence and conversion rates in addition to continuing exposure to tubercle bacilli. Among these might be mentioned cross-reactions, over-reading of the reactions, or variation of skin sensitivity in young children.

The facts that 61 per cent of the reactions were minimal (5 to 9 mm. induration) and that 3 per cent of the white children tested showed a substandard reaction suggest the possibility that some of the children classified as reactors might be reacting to some other organism, which might be antigenically related to the tubercle bacilli. Edwards and Palmer have shown that cross-reactions occur between PPD-S and PPD prepared from atypical acid-fast organisms of both the Kansas City and Battey strains (3). Tuberculin prepared from these organisms was not available for this study; consequently the effect of cross-reaction on the final results is unknown.

It is not believed that the factor of over-reading in the area of borderline positive reactions is of significance in this study. Despite the fact that during the ten-year period several different physicians performed and read the tests, the annual prevalence and conversion rates were remarkably constant.

The annual prevalence and conversion rates were recalculated, using 10 mm. induration as a dividing line between a reaction and no reaction. The results were lower but directly parallel to those obtained when an induration of 5 mm was used as a reaction.

The problem of cross-reaction as well as specificity of skin reactions and the sensitivity of the skin of children of this age group, as compared with that of children in the older age groups, offer some interesting points of speculation which can only be clarified by further study. This study is being continued among children of the eighth and ninth grades which will include many who participated originally in kindergarten and first grade. Preliminary data on one-year testing of 4,894 eighth- and ninth-grade white children indicate a prevalence rate of approximately 4 per cent. Obviously, this rate should be much higher if the

observed conversion rate of first-grade children had prevailed over the intervening years. Conversion or reversion rates for this older age group have not yet been established.

During the first five years of the study, histoplasmin sensitivity rates for white children were consistently higher than those for Negroes. By contrast, the conversion rates were consistently higher for Negroes during the entire ten-year study. These observed phenomena are not easily explained unless infection tends to occur at earlier ages among white children but occurs even more frequently among Negroes after three or four years of life, as shown by our kindergarten–first-grade conversion rates. This speculation, however, does not explain the equalization of prevalence rates during the last three years of the study.

Analysis of the yield of tuberculosis among the various contact groups is extremely interesting. Contrary to expectation, the case rate found among contacts of converters did not differ from that for contacts of index children who were tuberculin reactors when first tested.

As expected, the prevalence of positive tuberculin reactions was higher among Negro children than among white children. The difference was not nearly as striking for the ten-year study as the threefold difference reported from the survey of 1945 (4). The total yield of new tuberculosis among contacts of Negroes was somewhat higher than the corresponding yield among contacts of white children.

It has previously been shown that the size of the tuberculin reaction bears a definite relationship to the probability of the individual's developing active tuberculosis (5, 6). This study indicates that there is a direct relationship between the size of the tuberculin reaction of young children and the likelihood of the presence of active tuberculosis among the adult contacts.

From these studies one might set up certain priorities in this type of program, depending on the amount of funds available. Thus, both white and Negro children should be tested, since the yields are not markedly different in the two races. It would be preferable to test twice as many children on the first test rather than to re-test the nonreactors. Again, the household rather than the non-household contacts should be followed up and the follow-up should be scaled to the size of the reaction in the index child, giving highest priority to the contacts of the children with the largest reactions.

Summary

Observation of tuberculin and histoplasmin skin tests done yearly on kindergarten children in Kansas City for the past ten years are reviewed. During seven of these years re-tests of the nonreactors in the kindergarten were performed the following year in the first grade. Prevalence rates for tuberculin and histoplasmin are presented along with conversion rates for these two antigens for both the white and the Negro races. Follow-up study of the contacts of the tuberculin-positive children gave an index of the efficiency of this method in the finding of new cases of tuberculosis. Cost indices of this procedure are included. Some of the most important facts derived from this study are:

1. Tuberculin prevalence rates among children in the kindergarten

show no tendency to fall over the ten years of observation either in whites or Negroes.

2. The conversion rates to tuberculin observed in children who were nonreactors in kindergarten do not show a tendency to fall during the seven years of observation.

3. Use of the tuberculin test for obtaining index children whose contacts form a special risk group for extensive follow-up study not only gives a better yield of tuberculosis cases than mass roentgenography, but at the same time decreases the total exposure of a population to ionizing radiation.

4. The yield of active tuberculosis cases found by follow-up study of contacts of children who are converters was not greater than that found by follow-up study of children who were tuberculin reactors on the first test in kindergarten or first grade.

5. The cost of finding a case of tuberculosis by the methods of this study is approximately the same as for the mass roentgenographic method.

Sumario

Justipreciación del Descubrimiento de Casos de Tuberculosis por la Comprobación con Tuberculina en los Escolares Jóvenes

Repásanse los resultados de las pruebas con tuberculina e histoplasmina ejecutadas anualmente en las escuelas de párvulos de Kansas City durante los últimos diez años. Durante siete de esos años se verificaron recomprobaciones de los que no reaccionaban el año siguiente en el primer grado. Preséntanse los coeficientes de frecuencia para la tuberculina y la histoplasmina junto con los índices para ambos antígenos en los niños de raza blanca y raza negra. Un estudio subsiguiente de los contactos de los reactores a la tuberculina aportó un índice de la eficacia del método para el descubrimiento de nuevos casos de tuberculosis. Inclúyense datos del costo de este procedimiento.

Algunos de los datos más importantes derivados de este estudio son: (*1*) las tasas de positividad a la tuberculina en los niños de las escuelas de párvulos no revelaron en los diez años de observación tendencia a bajar ni en los sujetos de raza blanca o de raza negra; (*2*) las tasas de viraje en la reactividad a la tuberculina observadas en los niños que no reaccionaron en las escuelas de párvulos no revelaron tendencia a bajar en los siete años de observación; (*3*) el uso de la prueba de la tuberculina para descubrir niños cuyos contactos forman un grupo "amenazado especialmente" y deben ser objeto de extensa observación subsiguiente no sólo ofrece un rendimiento mayor de casos de tuberculosis que la radiografía colectiva, sino que a la vez disminuye la exposición total de una población a la irradiación yonizante; (*4*) el rendimiento en casos activos de tuberculosis descubiertos por el estudio subsiguiente de los contactos de niños que "viraron" después no fué mayor que el descubierto con el estudio subsiguiente de los contactos de niños que reaccionaron en la primera prueba ya en la escuela de párvulos o en el primer grado; (*5*) el costo de descubrir un caso de tuberculosis con los métodos usados en este estudio es aproximadamente el mismo que con la radiografía colectiva.

Resume

Une évaluation des résultats des enquêtes de dépistage de la tuberculose par le test à la tuberculine parmi les jeunes écoliers

Compte-rendu concernant l'observation de tests cutanés à l'histoplasmine et à la tuberculine pratiqués annuellement chez les écoliers des jardins d'enfants de Kansas-City

pendant ces dix dernières années. Au cours de sept de ces années, la répétition du test a été effectuée l'année suivante chez les sujets "négatifs" des jardins d'enfants quand ils étaient dans la classe suivante. Les taux prédominants pour la tuberculine et l'histoplasmine chez le Blanc et le Nègre sont présentés en même temps que les taux de virage pour ces deux antigènes. La poursuite des observations effectuées sur les sujets en contact avec les cas à réaction positive a fourni un indice de l'efficacité de cette méthode pour dépister les nouveaux cas de tuberculose. Les indices du coût de ce procédé sont indiqués.

Certains des faits les plus importants dérivés de cette étude sont: (*1*) la prédominance des taux concernant la tuberculine parmi les enfants des jardins d'enfants n'a montré aucune tendance à baisser au cours des dix années d'observation, soit chez le Blanc, soit chez le Nègre; (*2*) les taux de virage à la tuberculine observés chez les enfants à réaction négative, lorsqu'ils étaient dans le jardin d'enfants, n'ont pas montré de tendance à baisser durant les sept années d'observation; (*3*) l'usage du test à la tuberculine pour déceler les "enfants-indice" dont les contacts constituent un groupe "risque spécial" devant être suivi de façon extensive, non seulement permet de dépister plus de cas de tuberculose que la radiographie en masse, mais en même temps réduit la valeur totale de l'exposition d'une population aux radiations ionisantes; (*4*) le nombre de cas de tuberculose en activité, décelé par l'examen des sujets en contact avec ceux des enfants dont la cuti est devenue positive, n'était pas plus élevé que celui fourni par la poursuite de l'observation des cas "positifs" au premier test effectué au jardin d'enfants ou dans la classe suivante; (*5*) le coût du dépistage d'un cas de tuberculose par les méthodes utilisées dans cette étude est approximativement le même que celui du dépistage radiologique en masse.

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DYSPNEA AS A SYMPTOM IN PARKINSON'S SYNDROME[1, 2]

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INTRODUCTION

Parkinsonism can result in a wide variety of functional disabilities due to altered neurologic functions. Recently, a patient with parkinsonism was observed who complained of severe exertional dyspnea and in whom no pulmonary or cardiovascular basis for this complaint could be established.

V. B., a 37-year-old female, complained of dyspnea on mild exertion, of ten years' duration. At age twenty, the patient had an obscure febrile illness with clouding of consciousness. Following recovery, she remained well until age twenty-seven, when rigidity, tremors, slowness, and dyspnea on mild exertion developed. These symptoms progressed over the years. The physical examination at the time of this investigation revealed a vigorous-looking woman with rigidity of the face, trunk, and extremities and a four-per-second tremor involving the arms and hands. Many volitional acts were performed only after a delay of three to ten seconds. The physical examination was normal except for the neurologic abnormalities. An electrocardiogram was within normal limits. Chest fluoroscopy was normal with the exception of poor ability to carry out directions promptly and inability to hold breath for more than two seconds.

The observations on this patient stimulated a reappraisal of other individuals with parkinsonism, and 4 additional patients with similar symptoms were found.

Parkinson (1), in his description of the syndrome in 1817, reported that one of the patients "fetched his breath rather hard," but did not elaborate on this symptom. Denny-Brown (2), Grinker (3), Brain (4), Wilson (5), Baker (6), and Doshay (7) do not mention dyspnea as a symptom of this disease. Hypoventilation and dyspnea due to weakness of the respiratory muscles have been described in other neurologic and muscular diseases, particularly in poliomyelitis (8). Dyspnea has even been reported as a prominent presenting symptom in unusual cases of progressive muscular atrophy (9).

Respiratory disorders accompanying epidemic encephalitis were analyzed by Turner and Critchley (10) who stressed the frequency of intermittent or paroxysmal respiratory abnormalities after encephalitis. Except for respiratory insufficiency associated with bulbar nuclear involvement in acute encephalitis, they reported dyspnea only in association with paroxysmal apneic episodes.

Recently, respiratory studies in 7 patients with cerebellar ataxia and dyspnea have been reported (11). The cause for exertional dyspnea and diminished maxi-

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TABLE 1

CLINICAL STATUS

Subject	Sex	Age	Parkinsonism	Dyspnea	Rigidity	Tremor	Impaired Muscle Control	Incapacity
			(years)	(years)	(grade)	(grade)	(grade)	(grade)
J. P.	M	60	4	3–4	2	1	3	3
V. B.	F	37	10	10	2	3	3	3
E. K.	M	50	20	18	3	3	3	3
M. M.	F	42	15	*	1	2	3	3
M. S.	F	59	27	17	1	2	3	2
M. F.	M	62	20		1	2	1	1
E. L.	M	54	5		1	3	2	1
L. W.	F	46	32		3	1	2	1
J. B.	M	38	3		2	1	2	2

Duration in years of dyspnea refers to the time the patients have noted dyspnea with mild exertion. In grading rigidity, tremor, impaired muscle control, and incapacity, grade 1 indicates slight or none; grade 2, moderate; and grade 3, a marked degree of abnormality. Impaired muscle control includes difficulty in initiating actions, inability to perform some simple actions, and slow movement. Under the term incapacity, grade 1 indicates that the patient was able to carry out all of the usual activities of daily life without help from others, and grade 3 indicates he needed help both in dressing and in eating.

* *M. M.* was troubled by dyspnea on mild exertion only when she was not given her usual antiparkinsonian drugs.

mal breathing capacity in some of these cases was presumed to be ataxia of respiratory movements. In other cases, weakness of the respiratory muscles, pulmonary emphysema, or pulmonary tuberculosis may have contributed to the pulmonary insufficiency.

OUTLINE OF INVESTIGATION

Subjects: Five males and 4 females with Parkinson's syndrome were studied. In 3 patients (*L. W.*, *M. S.*, and *V. B.*), the history suggested encephalitis as the basis for parkinsonism, but in the remaining cases the etiology was not known.

The sex, age, duration of symptoms, and severity of the rigidity, tremor, impaired muscle control, and incapacity are listed in table 1. All of the patients were ambulatory, none appeared grossly enfeebled by the illness, and several had unusually good muscular development. Two patients were able to run 50 yards in seven seconds, which equals the performance of many normal persons.

Four of the 9 patients complained of dyspnea with mild exertion. Usually, this had begun one or two years after the onset of their parkinsonism and had progressed in severity along with the neuromuscular symptoms. Another patient (*M. M.*) had not noted dyspnea on exertion in the past but, when antiparkinsonian drugs were withheld during this study, she developed dyspnea on mild exertion.

OBSERVATIONS

Clinical Investigation

Exercise tolerance was tested by having the patient walk on level ground at a conventional pace (100 feet per fifteen to twenty-five seconds). Four patients

experienced no more respiratory discomfort at the end of five minutes of exercise than did the examiners. Five patients, however, developed labored breathing within one or two minutes after the onset of exercise. When encouraged to continue the exercise, they noted respiratory discomfort, and 2 complained of faintness and appeared acutely distressed.

There was no evidence obtained by history, physical examination, or electrocardiogram to suggest heart disease or chronic pulmonary disease. None of the patients was thyrotoxic, and there was no clinical suggestion of acidosis, although blood pH determinations were not carried out.

Chest fluoroscopy and postero-anterior chest roentgenograms revealed no abnormalities of the thoracic cage, pleurae, lungs, or heart. However, poor voluntary control of respiratory movements was noted; delay in initiating inspiration or expiration on command, inability to hold the breath for more than one or two seconds, and poor voluntary control over changes in the rate and depth of breathing were prominent abnormalities in 6 of the patients (the 5 dyspneic patients plus *E. L.*). These patients could perform some maneuvers rapidly, such as coughing or sniffing. No intrinsic tremors of the diaphragms were seen.

Respiratory investigations: Ventilatory functions were studied with a 13.5 liter Collins respirometer. The soda-lime cannister was removed to decrease resistance, and no attempt was made to measure tidal volumes accurately during quiet breathing. The maximal breathing capacity was determined by measuring the total expired air during a twelve- to fifteen-second period of maximal voluntary hyperventilation (12).

The maximal expiratory strength was determined with an ordinary mercury sphigmomanometer. The cuff and rubber bulb were removed and, in their place, a respirometer mouthpiece was connected by adaptors to the mercury column of the sphigmomanometer. With the mouthpiece in place, the subject expired as forcefully as possible for two seconds in order to maintain the mercury column at a maximal level. The results were expressed in millimeters of mercury. In measuring the maximal inspiratory strength, the mouthpiece was disconnected and reattached to the open end of the upright column of mercury. The test was performed with the patient inspiring maximally. In the case of *M. S.*, a tight fit with a mask or mouthpiece was not possible because of the distorted, fixed facial posture of the patient, and expiratory and inspiratory pressures could not be determined. Eight normal subjects, selected to correspond closely in sex, age, height, and weight with the patients, were similarly tested.

The gas exhaled during exercise was collected in a one-hundred-liter Douglas bag and measured in a respirometer. Two test situations were used. In the first, the patient was exercised by walking on level ground at a rate of 100 feet per fifteen to twenty-five seconds until mild dyspnea developed. When the patient began to experience dyspnea, or after two minutes of walking, the valve was opened for thirty seconds, and the expired air was collected in the Douglas bag while the patient continued to exercise at the same rate. In the second test, the patient walked or ran for one to five minutes until fairly severe dyspnea developed. The volume of expired air was similarly collected during the last thirty seconds of exercise.

Five subjects were given no antiparkinsonian drugs for three days before the studies. In the cases of *J. P.*, *V. B.*, *E. K.*, and *M. F.*, disability was so marked that it was not possible to discontinue the drugs.

The results of the pulmonary function studies are shown in table 2. The vital capacities were slightly reduced in most patients. Interestingly, some of the

TABLE 2

RESPIRATORY STUDIES

Patient	Dyspnea with Mild Exertion	Vital Capacity		Maximal Expiratory Effort	Maximal Inspiratory Effort	Maximal Breathing Capacity			Minute Ventilation		Breaths per Minute during Maximal Breathing Capacity
		Liters	Per Cent of Predicted			Measured	Predicted	Per Cent of Predicted	with Mild Exercise	with Severe Exercise	
				(mm. Hg.)	(mm. Hg.)	L./min.	L./min.		L./min.	L./min.	
J. P.	Yes	3.16	84	40	34	53	92	58	40	58	40
V. B.	Yes	2.31	80	25	20	49	93	53	31	54	45
E. K.	Yes	3.34	85	32	25	71	128	55	31	82	40
M. M.	Yes	2.63	97	38	36	54	81	67	30	60	45
M. S.	Yes	1.91	78			30	63	48	38	49	36
M. F.	No	4.76	129	50	40	97	105	92	38	59	65
E. L.	No	3.31	93	60	45	113	103	110	39	52	105
L. W.	No	2.71	100	65	67	83	88	94	33	50	120
J. B.	No	4.74	121	60	50	86	118	73	32	67	90
Average of all patients			96	46	39			72			
Average of nondyspneic patients			111	59	50			92			
Average of dyspneic patients			85	34	29			56			
Average of normal subjects				57	49						

The patients who developed dyspnea on mild exertion are indicated. The vital capacity is expressed as liters of air and as per cent of the predicted values. The maximal expiratory and inspiratory efforts are expressed in mm. of mercury. The maximal breathing capacity is expressed in liters of air per minute and as per cent of the predicted value. The minute ventilation with mild and with severe exercise is expressed in liters per minute. The timed collection periods for the maximal breathing capacities were 12 to 15 seconds, and for the minute ventilation with exercise, 20 to 30 seconds.

patients recorded larger vital capacities on spontaneous sighs than they did when breathing on command. The strength of inspiration and expiration, as measured by the technique indicated, was lower for the patients with parkinsonism than for the control group.

The mean value for the maximal breathing capacity in patients with dyspnea on mild exertion was 56 per cent of the mean predicted normal, whereas the mean value for the nondyspneic group was 92 per cent of the mean predicted normal.

No patient who developed dyspnea on mild exercise was able to attain a rate above 45 breaths per minute during the maximal breathing capacity test. A typical respirometer tracing from one of these patients is shown in figure 1*A*. It should be noted that, on the command to begin maximal hyperventilation, only a slight change in the rate or the depth of respiration occurred.

One of the 4 patients who did not become dyspneic on mild exertion had a maximal breathing capacity of 73 per cent of the predicted normal. A part of the maximal breathing capacity tracing from this patient is shown in figure 1*B*. On

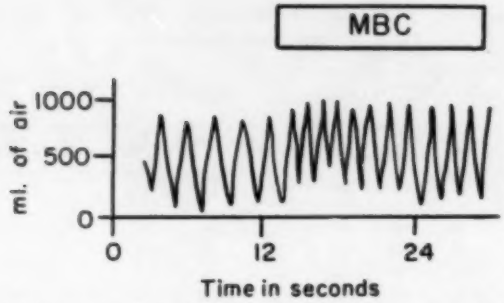

FIG. 1*A*. Part of the respirogram of patient *V. B.*, with the patterns before and after the patient was asked to begin her maximal breathing capacity test.

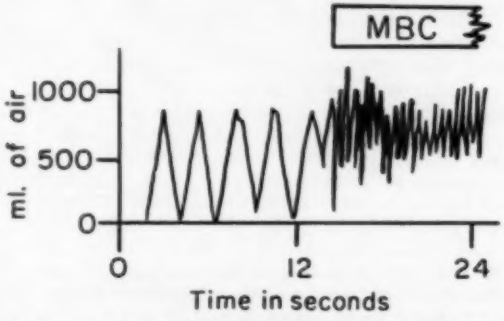

FIG. 1*B*. Part of the respirogram of patient *J. B.*, with the patterns before and after the patient was asked to begin his maximal breathing capacity test.

TABLE 3

RESPIRATORY STUDIES WITH AND WITHOUT DRUG THERAPY

Patient	Vital Capacity Per Cent of Predicted Normal		Maximal Breathing Capacity, Per Cent of Predicted Normal		Breaths per Minute during Maximal Breathing Capacity	
	Before Drugs	After Drugs	Before Drugs	After Drugs	Before Drugs	After Drugs
M. M.	97	130	67	98	45	95
M. S.	78	87	48	101	36	80

beginning maximal hyperventilation, the rate increased to ninety breaths per minute, but the tidal volume was small. Altering the rate and depth of breathing in various trials failed to increase the maximal breathing capacity.

During exercise to the point of mild dyspnea, the minute ventilation in several patients was almost equal to the measured maximal breathing capacity. In all 5 dyspneic patients, exercise to the point of severe dyspnea resulted in a minute ventilation exceeding the resting maximal breathing capacity.

Five patients were studied while they received no antiparkinsonian drugs. Two of these had very low maximal breathing capacities which increased markedly after drug therapy was resumed. While on drug therapy, both patients received 5 mg. of trihexyphenidyl hydrochloride (Artane®) three times a day orally and, in addition, patient *M. S.* received 50 mg. of diphenhydramine hydrochloride (Benadryl®) orally three times a day. Both patients took no drugs for three days before the nontreatment part of the study. These results are shown in table 3.

Discussion

The dyspneic and nondyspneic groups do not differ strikingly in age, sex distribution, duration of parkinsonism, or degree of rigidity or tremor, as seen in table 1. However, it is the patients with the greatest impairment of muscle control and the most marked incapacity in terms of the activities of daily living who tend to have dyspnea with mild exertion.

Respiratory insufficiency may be caused by defects of gas exchange, by inadequate ventilation, or by both (12). There was no evidence of an alveolar-capillary diffusion defect. Since the limited maximal breathing capacity gave evidence of definite ventilatory deficiency, the investigations were confined to studies of ventilation.

Ventilatory insufficiency may be due to either obstructive or restrictive defects.

Neither physical examination, chest fluoroscopy, or examination of the spirograms presented evidence to suggest obstruction to air flow in the parkinsonian patients. In figure 2, a portion of the spirogram of *E. K.* (including the vital capacity recording), it is demonstrated that the patient was able to expire a large percentage of his total vital capacity very rapidly. The vital capacity tracings of the other patients were similar.

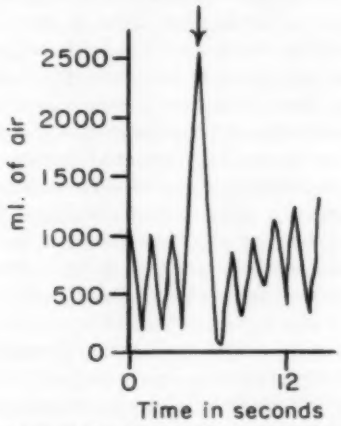

Fig. 2. Part of the respirogram of patient *E. K.* An attempted vital capacity is marked with an arrow.

Restrictive defects can be divided into those associated with abnormalities of the lungs, the thoracic bellows, and the nervous system. No anatomic abnormalities were observed in the lungs or the thoracic cage. However, dynamic abnormalities such as rigidity or weakness of the diaphragm, chest wall muscles, or other muscles of respiration may contribute to a restrictive defect.

It is not possible to evaluate quantitatively the role of rigidity of the chest wall without extensive analyses of chest wall mechanics. The patients could at times perform respiratory movements very quickly, as seen by clinical, fluoroscopic, and spirometric observation. A fairly high minute ventilation was achieved by some of the dyspneic patients during vigorous exercise. This suggests that increased rigidity is not a significant factor or, if it plays a role, it is not present at all times to the same degree.

The slightly low vital capacity determinations may be accounted for by difficulty in holding a mouthpiece securely, inability to initiate voluntary movements, and other factors related to Parkinson's syndrome, rather than to limitations of lung volumes. The depth of breathing during the performance of the maximal breathing capacity test was only a small portion of the vital capacity. This mild reduction of the vital capacity would not be expected to affect the maximal breathing capacity (13) or to produce dyspnea.

Comroe (14) states that a normal subject can develop 60 to 100 mm. of mercurial pressure on maximal expiration or inspiration. In the present studies, expiratory and inspiratory pressures of this magnitude were frequent among medical students and young physicians; however, students and physicians tend to have unusually high motivations in many test situations. The control group referred to in table 2 was selected from a hospital outpatient clinic. The composition of the control group for this study may account in part for the low mean values in the normal group as compared with those usually cited. However, the values determined in the parkinsonians were even lower than those of the control group. Difficulties similar to those noted in the vital capacity determinations appeared during the measurement of expiratory and inspiratory strength.

Abnormal neural mechanisms might be expected to contribute to a restrictive defect in these patients, since alteration of neuromuscular function is the basic abnormality in Parkinson's disease. Weakness may play some role, as discussed above. However, minute ventilations achieved during severe exercise in the dyspneic patients were greater than the maximal breathing capacities determined at rest; simple muscle weakness would not explain this phenomenon satisfactorily.

The dyspneic patients manifested the most marked impairment of motor control and the greatest incapacity in terms of the acts of daily living. There was good correlation of the maximal breathing capacity with ventilatory rate during performance of the test (*see* table 2). Two of the patients were studied while receiving no antiparkinsonian drugs. At that time the maximal breathing capacity was low, as was the ventilatory rate during its performance. After resumption of treatment they were able to breathe at a much faster rate and to achieve a maximal breathing capacity approaching the predicted normal (*see* table 3).

Figure 1*A* is representative of the many maximal breathing capacity tests carried out on the dyspneic patients. Repeated trials to improve the performance failed to modify significantly the maximal breathing capacity.

The striking reduction in the maximal breathing capacities of the patients was correlated with dyspnea; the 5 patients with dyspnea on mild exertion had a mean maximal breathing capacity of 56 per cent of the predicted normal, whereas the nondyspneic group had a mean maximal breathing capacity of 92 per cent of the predicted normal.

In all of the 5 dyspneic patients, exercise to the point of severe dyspnea gave minute ventilations exceeding the maximal breathing capacity determined at rest. The normal subject, when exercised to the point of severe dyspnea, usually achieves a minute ventilation in the range of 70 per cent of the maximal breathing capacity (14). The contrast between the high minute ventilation achieved during severe exercise and the limited maximal breathing capacity determined at rest suggests that their ventilatory abnormality is not a simple mechanical restrictive defect.

Nonrespiratory factors may contribute to the dyspnea observed in these patients. Observation of the patients walking on level ground suggested that the tremor, rigidity, and abnormal gait required a greater energy expenditure than in the normal person. The relatively high minute ventilation on walking also suggested that a high energy requirement was present, although oxygen consumption during exercise was not measured.

Another possible basis for dyspnea in these patients is poor muscle training. However, walking at a normal rate on level ground was not an unaccustomed exercise for these patients but one which all of the patients performed daily.

Summary

Respiratory studies on 9 patients with Parkinson's syndrome are presented. Five patients had dyspnea on mild exertion. This symptom occurred in patients with the most impaired muscle control and in those who were most incapacitated by their neurologic disease. No primary cardiac, pulmonary, or systemic disease other than the parkinsonism could be found to explain the respiratory insufficiency. The maximal breathing capacity of the 5 dyspneic patients was, on the average, 56 per cent of the predicted normal. This marked decrease in the mean maximal breathing capacity may, in part, have been the result of decreased respiratory strength and increased rigidity of the chest wall. A decreased ability to perform rapid, repetitive movements and poor muscle control were associated with the respiratory defect. Two subjects had greater maximal breathing capacities while receiving drugs than they had had while untreated. All of the dyspneic patients were able to achieve higher minute ventilations on severe exertion than during the performance of the voluntary maximal breathing capacity at rest. Pulmonary function studies offer an objective measure of part of the neurologic impairment in Parkinson's disease, and may be useful in following responses to medical or surgical therapy.

Sumario

La Disnea como Síntoma en la Enfermedad de Parkinson

Preséntanse estudios respiratorios ejecutados en 9 enfermos que padecían del síndrome de Parkinson. Cinco de ellos experimentaban disnea a un esfuerzo leve. Este síntoma aparecía en los que tenían más afectado el dominio muscular y en los más incapacitados por su afección nerviosa. Aparte del parkinsonismo, no pudo descubrirse ninguna dolencia cardíaca, pulmonar, o constitucional primaria que explicara la insuficiencia respiratoria. La capacidad respiratoria máxima de los 5 sujetos disneicos representó, en conjunto, 56 por ciento de la cifra normal predicha. Este notable disminución de la capacidad respiratoria máxima media tal vez fuera la consecuencia en parte de la menor potencia respiratoria y la mayor rigidez de la pared torácica. Con el defecto respiratorio se asociaban una disminución de la capacidad para ejecutar rápidos movimientos repetidos e imperfecto dominio muscular. Los sujetos mostraron mayor capacidad respiratoria máxima mientras recibían medicación que la desplegada mientras estaban sin tratar. Todos los disneicos pudieron ejecutar más aireaciones por minuto al esfuerzo intenso que durante la ejecución de la capacidad respiratoria máxima voluntaria en reposo. Los estudios de la función pulmonar aportan una medición objetiva del deterioro nervioso en la enfermedad de Parkinson y pueden resultar útiles para determinar la respuesta a la terapéutica médica o quirúrgica.

Resume

La dyspnée au titre de symptôme dans la maladie de Parkinson

Des études respiratoires pratiquées chez 9 malades atteints de maladie de Parkinson sont présentées. Chez 5 malades, la dyspnée survenait au moindre effort. Ce symptôme se manifestait chez ceux dont le contrôle musculaire était le plus altéré et ceux dont l'invalidité due aux lésions neurologiques était la plus prononcée. En dehors du parkinsonisme, l'insuffisance respiratoire ne pouvait être imputée à aucune maladie primitive cardiaque, pulmonaire, ou organique. La capacité respiratoire maximale des 5 malades dyspnéiques était, en moyenne, de 56% par rapport à la normale prévue. Cette réduction marquée de la capacité respiratoire maximale moyenne peut, en partie, résulter d'une réduction de la force de la respiration et de l'augmentation de la rigidité de la paroi thoracique. Une réduction de la capacité d'exercer des mouvements rapides répétés et un contrôle musculaire déficient étaient associés au défaut respiratoire. Chez 2 sujets, la capacité respiratoire maximale était plus élevée durant une thérapeutique médicamenteuse que dans une période sans traitement. Les valeurs de la ventilation-minute présentées par les malades dyspnéiques soumis à un effort sévère étaient plus élevées que durant l'épreuve de la capacité respiratoire maximale volontaire au repos. L'étude de la fonction pulmonaire offre une mesure objective d'une partie de l'altération neurologique dans la maladie de Parkinson et elle peut être utile pour évaluer la réponse au traitement médical ou chirurgical.

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THE VALUE OF AN INDEX OF MAXIMAL EXPIRATORY FORCE IN THE INTERPRETATION OF TESTS OF MAXIMAL VENTILATORY CAPACITY[1]

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With the technical assistance of Lilian Malkin, Meryl Lennox, and Jeanette O'Donovan

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Introduction

Indices of ventilatory capacity measure either the quantity of air that can be inspired or expired over a ten- to thirty-second period, or depend upon the velocity of a single expiration or a part thereof. An example of the former type is the maximal breathing capacity (or maximal voluntary ventilation) (1), while indices of the latter type include the timed vital capacity (2) and the maximal mid-expiratory flow rate (3).

Although factors such as lung compliance play a part, all of these indices depend principally upon two major variables, the nonelastic resistance of the lungs and the power of the chest available for ventilation. Although the theoretic dependence of ventilatory tests upon both of these factors has been widely acknowledged, there has been a tendency, at least on the part of clinical investigators, to equate the index of ventilatory capacity with respiratory resistance or airway patency. Were the power of the chest bellows assessed at the same time, the result of the test of ventilatory capacity would theoretically have more meaning as an index of resistance.[3] The study described below was designed to determine to what extent this reasoning was correct.

Methods

Two measurements of ventilatory capacity were selected for study. They were the maximal mid-expiratory flow rate (MMF) and the maximal breathing capacity (MBC). The former measurement was performed on "Lode" or "Pulmotest" spirometers with bell weights of 248 gm. and 258 gm., respectively, at paper speeds of 20 mm. per second. The MBC was measured over fifteen seconds, using either of the above or Benedict-Roth type of spirometers with soda-lime removed. An index of the power of the chest bellows was obtained by measuring the maximal expiratory pressure (MEP) obtainable following a maximal inspiration (4). A 15-gauge hypodermic needle perforating the tube leading to the mercury manometer provided a leak which made it impossible for the test to be performed with the glottis closed. The highest pressure that could be maintained for one to two seconds in three trials was accepted as the MEP. For the purpose of this study, expiratory resistance (cm. H_2O per liter per second) was measured by comparison of instantaneous esophageal pressure (5) with flow at the point during a quiet expiration at which pressure to overcome nonelastic resistance was greatest. The values recorded are mean figures based

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[3] This point was first brought to the writer's attention by Dr. Jere Mead.

on six breaths. Flow was measured either with a Lilly pneumotachograph or by observing the tangent to an instantaneous volume record obtained from a "pressure box" spirometer (6).

Ninety-nine adult subjects of both sexes were subjected to these three tests during routine study in two adjacent laboratories. Clinically, they varied from normal, healthy recruits of the mining industry, through all degrees of pulmonary disability, to advanced obstructive and obliterative pulmonary disease.

Results

The degree of correlation between the ventilatory tests, maximal breathing capacity (MBC), and maximal mid-expiratory flow (MMF), and the measured resistance was first investigated. Both the MBC and the MMF were related significantly to the expiratory resistance ($r = -0.43$ and -0.45 respectively; P = less than 0.001) (table 1). As may be seen from the table, both of the ventilatory tests were also significantly related to the maximal expiratory pressure (MEP).

On physical principles, flow is related to the ratio of pressure drop to resistance. Flow, as reflected by the values for MBC and MMF in this instance is an index of the maximal flow achievable under two different sets of circumstances. The pressure drop (alveolus to atmosphere) existing during the maximal ventilation tests is unknown, but may be expected to be related to the maximal expiratory pressure which can be developed under static conditions. The resistance existing during the performance of the ventilatory tests is likewise unknown and is a value which will vary from instant to instant. However, it is not unlikely that a hypothetic mean value will relate closely to the resistance during quiet breathing as measured in this study. With this in mind, correlation was sought between the ventilatory tests (MBC and MMF) on the one hand and the ratio of maximal expiratory pressure (MEP) to resistance on the other. This was found to be higher ($r = +0.59$) than either of the previous correlations observed.

From these correlation coefficients, regression equations were evolved describing the relationship of measured nonelastic resistance to the ratio of MEP over MBC and MMF.

TABLE 1

Correlation Coefficients Relating Maximal Breathing Capacity and Maximal Mid-expiratory Flow to Each Other and to Maximal Expiratory Pressure, Expiratory Nonelastic Resistance, and the Ratio of Maximal Expiratory Pressure to Resistance

	Maximal Mid-expiratory Flow	Resistance	Maximal Expiratory Pressure	Maximal Expiratory Pressure / Resistance
Maximal breathing capacity	+0.6	−0.43	+0.42	+0.59
Maximal mid-expiratory flow	—	−0.45	+0.23	+0.59

All correlations are highly significant ($P = < 0.001$) except for the correlation of MMF and MEP ($r = +0.23$, $P = 0.02$).

Thus: $R = \frac{MEP}{6.1\,MMF + 8.11}$ and $R = \frac{MEP}{0.145\,MBC + 4.36}$

Where: R = Nonelastic respiratory resistance, cm. H_2O per liter per second
MEP = Maximal expiratory pressure, mm. of mercury
MMF = Maximal mid-expiratory flow, liters per second
MBC = Maximal breathing capacity, liters per minute

From the above correlations it can be concluded that the measured expiratory resistance of a subject is indicated more accurately by using a regression equation based on the MEP and the ventilatory test (MBC or MMF) than it can be by either of the ventilatory tests alone.

The extent to which this is true can be illustrated in the following way: If the value of 3 cm. of water per liter per second is arbitrarily accepted as the upper limit of normal resistance, it is found that 33 subjects had resistances below this value, and 66 above. If the figure of 3 liters per second is also arbitrarily accepted as the lower limit of normal MMF, a raised resistance is correctly predicted in 56 of 66 cases by the MMF test, and in 52 of 66 cases using the predicted resistance based on the regression equation. However, the MMF indicates normal resistance in only 9 of 33 subjects with a measured normal resistance, whereas the predicted value, based on the regression, indicates normality in 27 of these 33 subjects.

Discussion

The ability of a subject to breathe at high velocities depends upon several factors. Some, such as volition, skill, and coordination, are incapable of measurement. Others, such as the muscle power available for breathing and the nonelastic resistance of the lungs can be assessed with some accuracy. Attempts have previously been made to establish correlation individually between the results of ventilatory tests and these latter two functions. Matheson and associates (4) found no correlation, however, between the maximal breathing capacity (MBC) and the maximal expiratory pressure (MEP) in normal subjects, while an absence of correlation was found in young miners by Carpenter and associates (7). The latter did, however, establish a correlation of rather low significance between the MBC and airway resistance. It is most likely that the failure of these workers to establish significant correlations between functions which logically ought to correlate was due to the fact that all of their subjects were "normal." When the range of data is extended from normal to severe impairment of function, as was done here, these factors appear to correlate with a high degree of significance. Furthermore, as resistance is a function of pressure gradient over flow, the relationship showing highest correlation would be one which took account of all three variables. That this is factually so was demonstrated in this study.

However, the potential advantages of using a regression equation based on the MBC or the MMF and the test of expiratory power are reduced by the very introduction of a further maneuver, depending on cooperation and skill. All of the

tests in this study were carried out in two entirely separate laboratories by several workers, and the population tested included many mine workers with compensation claims, from whom maximal cooperation could not always be expected. In several instances, a MEP of 10 or 15 mm. of mercury was obviously fallacious in an apparently powerful and healthy individual, and values such as these are clearly responsible for a wide divergence of the predicted from the actual resistance in some cases. Furthermore, the techniques by which some of these measurements were obtained are undoubtedly capable of improvement. Measurements of the MBC were made on three different types of instrument, all of which have been demonstrated to be inaccurate for very high values (8). However, this was probably not a major source of error as the majority of the values recorded were normal or low, only 13 subjects producing MBC values in excess of 170 liters per minute.

In conclusion it may be said, however, that even bearing these factors in mind, a predicted resistance based both on the MBC or the MMF and the MEP gave a better indication of the nonelastic resistance than either of the ventilatory tests alone. It is suggested that the inclusion of a test of maximal expiratory power sufficiently increases the information obtainable from tests of maximal ventilation to justify its routine inclusion in clinical lung function testing.

Summary

In testing 99 adult subjects, a significant correlation was found between tests of maximal ventilatory capacity on the one hand, and both the expiratory resistance and the power of the chest, on the other.

Expiratory resistance can thus be predicted with reasonable accuracy from measurements of maximal breathing capacity or maximal mid-expiratory flow rate, and the maximal expiratory pressure which can be developed by the thoracic cage.

It is suggested that the expiratory resistance predicted in this way is more informative than either the value for maximal breathing capacity or the maximal mid-expiratory flow rate alone.

Sumario

El Valor de un Indice de la Fuerza Espiratoria Máxima en la Interpretación de la Capacidad Ventiladora Máxima

Al comprobar a 99 sujetos adultos, se descubrió una correlación importante entre las pruebas de la capacidad ventiladora máxima y las de la resistencia espiratoria y la potencia del tórax.

Cabe así predecir la resistencia espiratoria con bastante exactitud por las mediciones de la capacidad respiratoria máxima o la velocidad de la corriente meso-espiratoria máxima y la presión espiratoria máxima que puede crear la caja torácica.

Señálase que la resistencia espiratoria predicha de este modo es más informativa que el valor de la capacidad respiratoria máxima o el de la velocidad de la corriente meso-espiratoria máxima, tomados por sí solos.

Resume

La valeur d'un indice de force expiratoire maximale dans l'interprétation des tests de la capacité ventilatoire maximale

Chez 99 adultes testés, une corrélation significative a été observée entre les tests de la capacité ventilatoire maximale, les tests de la résistance expiratoire, et de la force de la cage thoracique.

La résistance expiratoire peut ainsi être prévue avec suffisamment de précision en se fondant sur les déterminations de la capacité respiratoire maximale ou du taux du débit "mid-expiratory" maximal et de la pression expiratoire maximale que peut exercer la cage thoracique.

L'auteur estime que la résistance expiratoire ainsi évaluée donne plus de renseignements que la valeur de la capacité respiratoire maximale ou celle du débit "mid-expiratory" seul.

Acknowledgments

The stimulus to this study provided by Dr. Jere Mead of the Department of Physiology of the Harvard School of Public Health is gratefully acknowledged.

Dr. M. R. Becklake of the Pneumoconiosis Research Unit of the Council for Scientific and Industrial Research supplied much of the data and assisted in their analysis.

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FULL-TERM DELIVERY FOLLOWING MAJOR THORACIC SURGERY FOR TUBERCULOSIS[1,2]

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INTRODUCTION

In the past decade the treatment of tuberculosis has changed as a result of antimicrobial therapy and thoracic surgery. In a previous paper on the treatment of tuberculosis and pregnancy, the present writers pointed out that 37 per cent of their patients in the past five years (1952–1956) had received antimicrobial drugs, compared with 1 per cent in the years 1933–1951 (1). The present paper is an analysis of the patients subjected to major thoracic surgery who were delivered at The New York Lying-In Hospital from January 1, 1952, through December 31, 1956. Eleven per cent of the patients delivered during this period had major thoracic operations before or during pregnancy compared with 2.3 per cent in the preceding years.

Review of the Literature

In 1929, Jesson (2) first described the result of pregnancy following thoracoplasty. Folsome and Kuntze (3) in 1953 were able to collect 119 cases in which thoracoplasty was performed; 34 during pregnancy and 85 before pregnancy. They reported an additional 25 patients who had the following major thoracic operations associated with pregnancy: two lobectomies; fourteen pneumonectomies; nine three-stage thoracoplasties. Two of the pneumonectomies were done during pregnancy as were two of the thoracoplasties.

Holden and associates (4) in 1954 reported the cases of 10 patients on whom major thoracic operations were performed during pregnancy; 5 had thoracoplasties between the third and sixth months of gestation, and 5 had resectional surgery (three lobectomies, one pneumonectomy, and one segmental resection) between the second and sixth months of gestation.

Corner and Nesbitt (5) reported the cases of 15 patients from the Johns Hopkins Hospital in whom pregnancy was related to pulmonary resection. Only 3 of these patients were operated on for tuberculosis; one patient had a lobectomy and 2 had thoracoplasties. Of the remaining 12 patients, 10 had bronchiectasis and 2 had bronchial adenoma.

Murdock (6) in 1955 analyzed 15 patients, 14 of whom had normal deliveries at term and one a spontaneous abortion. All of these had had major thoracic surgery four and one-half to nine years prior to conception. Of these 15 patients, 3 had pneumonectomies; 5, lobectomies; 5, segmental resections; and 2, thoracoplasties. In that series there were no cases of reactivation of disease during pregnancy or from twelve to thirty-five months after conception. No treatment of any kind was given during pregnancy or in the puerperium.

Foley and associates (7) collected from the literature 62 cases of pulmonary resection prior to or during pregnancy, 41 of which were done for pulmonary tuberculosis. Fifteen operations were done during pregnancy and 47 before conception.

[1] From the Department of Obstetrics and Gynecology of the Cornell-University Medical College and the Woman's Clinic of The New York Hospital, New York 21, New York.

[2] Presented before the Medical Session, as part of *Section 1A*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 19, 1958.

TABLE 1

SUMMARY OF FULL-TERM DELIVERIES FOLLOWING MAJOR THORACIC SURGERY FOR TUBERCULOSIS

Case Number	Onset of Symptoms	Type of Disease	Thoracic Operation	Date of Operation	Type of Delivery	Date of Delivery	Activity of Tuberculosis During Pregnancy	Follow-Up Study After Delivery	Result
								months	
1	1947	Far advanced	Right pneumonectomy Right thoracoplasty	June, 1948 July, 1948	Low-forceps	February, 1955	Inactive	26	Delivered again, March, 1958
2	1947	Far advanced	Right thoracoplasty	June, 1948	Normal spontaneous delivery	March, 1955	Inactive	23	No change
3	1947	Moderately vanced	Segmental resection right upper lobe	December, 1950	Mid-forceps	April, 1952	Inactive	33	No change
			Right thoracoplasty	January, 1951	Low-forceps	September, 1954	Inactive	18	No change
4	1947	Far advanced	Right upper lobectomy	March, 1949	Normal spontaneous delivery	July, 1952	Inactive	24	No change
5	1948	Far advanced	Left upper lobectomy	September, 1951	Normal spontaneous delivery	May, 1952	Active	70	Improved; delivered again, June, 1957
6	1941	Far advanced	Right upper lobectomy	January, 1952	Low-forceps	September, 1952	Active	48	Improved; delivered again, 1954
7	1938	Far advanced	Left thoracoplasty	1939	Low-forceps	January, 1952	Inactive	73	No change; delivered again, July, 1957

8	1951	Moderately advanced	Segmental resection, left upper lobe	January, 1953	Breech extraction	June, 1954	Inactive	15	No change
9	1954	Far advanced	Left upper lobectomy Thoracoplasty	August, 1955 August, 1955	Normal spontaneous delivery	May, 1955	Active	33	Improved; delivered again, April, 1957
10	1946	Moderately advanced	Left extrapleural plombage	May, 1952	Low-forceps	March, 1955	Active	13	Improved
11	1952	Moderately advanced	Segmental resection, right upper lobe	October, 1953	Normal spontaneous delivery	June, 1955	Inactive	31	No change; delivered again May, 1957
12	1948	Far advanced	Left upper lobectomy Right upper lobectomy Right thoracoplasty	1949 1950 1951	Low-forceps Low-forceps	June, 1953 December, 1956	Inactive Inactive	55 15	No change No change
13	1952	Far advanced	Segmental resection, left upper lobe	April, 1953	Normal spontaneous delivery	October, 1956	Inactive	16	No change
14	1953	Moderately advanced	Right upper lobectomy	April, 1953	Normal spontaneous delivery	June, 1956	Inactive	12	No change
15	1952	Minimal	Segmental resection, right upper lobe (tuberculoma)	December, 1952	Normal spontaneous delivery	June, 1955	Inactive	32	No change
16	1938	Moderately advanced	Right upper lobectomy	July, 1951	Low-forceps	September, 1955	Inactive	27	No change

TABLE 1—CONTINUED

Case Number	Onset of Symptoms	Type of Disease	Thoracic Operation	Date of Operation	Type of Delivery	Date of Delivery	Activity of Tuberculosis During Pregnancy	Follow-Up Study After Delivery	Result
								months	
17	1954	Moderately advanced	Right upper lobectomy	June, 1954	Normal spontaneous delivery	November, 1956	Inactive	12	No change
18	1953	Moderately advanced	Left upper lobectomy	July, 1954	Low-forceps	September, 1956	Inactive	13	No change
19	1936	Far advanced	Right thoracoplasty Left thoracoplasty	1947 1947	Low-forceps	January, 1956	Inactive	24	No change; delivered again, September, 1957
20	1948	Far advanced	Segmental resection, right lower lobe Right upper lobectomy	July, 1951 November, 1951	Cesarean section	December, 1956	Inactive	12	No change
21	1943	Moderately advanced	Right thoracoplasty	1947	Normal spontaneous delivery	August, 1953	Inactive	39	No change

22	1947	Moderately advanced	Left upper lobectomy	October, 1951	Low-forceps	October, 1953	Inactive	27	No change
23	1946	Moderately advanced	Right thoracoplasty	January, 1950	Low-forceps	October, 1952	Inactive	48	No change
24	1948	Moderately advanced	Left thoracoplasty	1949	Mid-forceps Normal spontaneous delivery	June, 1952 November, 1953	Inactive Inactive	18 1	No change Progressed
25	1946	Moderately advanced	Right upper lobectomy Right thoracoplasty	April, 1953 May, 1953	Breech extraction	August, 1953	Active	55	Improved; delivered again, February, 1958
26	1947	Moderately advanced	Right thoracoplasty	November, 1947	Low-forceps	August, 1953	Inactive	24	No change
27	1949	Far advanced	Left upper lobectomy	April, 1950	Low-forceps	May, 1953	Inactive	5	Progressed
28	1948	Moderately advanced	Right upper lobectomy	September, 1952	Normal spontaneous delivery	September, 1954	Inactive	16	No change
29	1941	Moderately advanced	Left thoracoplasty	1942	Normal spontaneous delivery	March, 1952	Inactive	50	No change

Material and Methods

From January 1, 1952, to December 31, 1956, there were 298 deliveries in 257 patients with pulmonary tuberculosis at The New York Lying-In Hospital; 29 patients who had 32 deliveries were subjected to major thoracic operations before or during pregnancy. (There have been 7 additional full-term deliveries in these patients since January 1, 1957.) With the exception of a few private patients delivered by other members of the staff, all of the remainder were under the direct supervision of the present writers.

Patients with inactive tuberculosis are seen in the Obstetric-Pulmonary Clinic of this hospital on an average of every two weeks during the first seven months of gestation and every week thereafter. At least three sputum or gastric analyses are done during gestation and one immediately after delivery. A phthisiologist examines all of the patients during their antepartum course. Chest roentgenograms were formerly obtained every two or three months, but are now obtained when the patient first registers for delivery and in the last trimester. A postpartum roentgenogram was formerly taken one week following delivery and three months post partum. At present the film is frequently omitted immediately following delivery.

Pregnant patients with active pulmonary tuberculosis are admitted to the obstetric floor where facilities are available for both obstetric and medical care. Medical care is carried out in conjunction with the phthisiologist, and the patient is treated for her tuberculosis as is the nonpregnant patient. The only exception to this is that major surgical procedures are not performed on the pregnant patient in the last two months of gestation. Antimicrobial therapy is continued, and operation is delayed until six weeks or longer after delivery.

Table 1 is a summary of the surgical and obstetrical procedures performed on the patients included in this study.

The type and extent of tuberculosis during pregnancy is indicated in table 2.

Five patients had active tuberculosis during gestation. Three of these patients were operated upon during gestation; the fourth was operated upon shortly after delivery; and the fifth had been operated upon three years before conception. This last patient had had sputum intermittently positive for tubercle bacilli for eight years prior to delivery. Since

TABLE 2

Type and Extent of Tuberculosis During Pregnancy

	Active	Inactive
Minimal	0	1
Moderately advanced	4	15
Far advanced	1	11
Total	5	27

TABLE 3

Type of Operation

Pneumonectomy	1
Lobectomy	15
Segmental resection	6
Thoracoplasty (primary)	9
Thoracoplasty with other procedures	6
Extrapleural plombage	1
Total	38

infectious sputum was present during pregnancy, she was considered to have active disease and was treated with antimicrobial therapy. Of the 24 patients with inactive disease, who had 27 deliveries, the disease in 2 patients became active after delivery. These patients will be discussed below.

The various types of thoracic operations performed on the patients are listed in table 3.

Thirty-eight operative procedures were performed either as separate operations or in conjunction with thoracoplasty; thoracoplasty was performed four times after lobectomy, once after pneumonectomy, and once after segmental resection. Several patients had bilateral operations performed at different times. Nine thoracoplasties were done as primary procedures before 1950. With the advent of chemotherapy and excisional surgery, thoracoplasty has been utilized only as a secondary procedure. Lobectomy was the most frequent procedure, and this operation was done in 15 instances.

With the exception of the 3 patients operated upon during pregnancy and the one patient shortly after delivery, the remaining operations were performed from 1.5 to 13 years before delivery; the average time was 4.3 years.

Antimicrobial Therapy in Relation to Surgery and Pregnancy

All of the patients who had excisional surgery performed after 1949 received antimicrobial drugs before or after surgery. A few patients received streptomycin before operation when this was the only drug available. The majority of the patients received streptomycin plus PAS and/or isoniazid both before and after operation. In 6 patients who had primary thoracoplasties between 1939 and 1950, no chemotherapy was employed; the remaining 3 patients who had primary thoracoplasties received streptomycin for several months, either before or after operation.

Antimicrobial therapy was employed during pregnancy in 7 patients; 5 were considered to have active tuberculosis; 2 other patients were receiving antimicrobial therapy before conception, and this was continued during pregnancy.

Management of Labor and Delivery

Analgesia and anesthesia: In the 32 deliveries which occurred in the 29 patients who had major thoracic surgery, no analgesia was used in 5 instances, pentobarbital and/or meperidine (Demerol®) was given to 26 patients, and meperidine plus scopolamine were given to one patient. The present writers prefer to use pentobarbital and meperidine in tuberculous patients. Narcotic doses of drugs which depress the cough reflex and voluntary expectoration should be avoided. Anesthesia used in these patients was local and/or pudendal block in 19 patients, several of whom received whiffs of nitrous oxide for a few moments at delivery; 5 patients were given cyclopropane; 5 received small amounts of gas-oxygen; and 3 did not require anesthesia. Whenever possible, local anesthesia is recommended for delivery of the tuberculous patient.

TABLE 4

TYPE OF DELIVERY

Normal spontaneous delivery	13
Low-forceps	14
Low mid-forceps	2
Breech	2
Cesarean section	1
Total	32

Type of delivery: The type of delivery used for the patients in the present study is described in table 4.

It is the writers' practice to permit normal spontaneous delivery if labor is progressing satisfactorily and if the second stage is not long or tiring. Forty per cent of the patients who had major thoracic operations had normal spontaneous deliveries. Forceps were used to shorten the second stage of labor in 56 per cent of the surgical group, compared with 31 per cent in the nonsurgical group previously reported (1). One cesarean section was done for obstetrical indications.

The management of the puerperium in the tuberculous patient on this service differs from that in the nontuberculous woman and has been described elsewhere (8, 9). Slower ambulation is advised as well as a longer period of rest, and breast feeding is not permitted. If antimicrobial drugs have been given during gestation, they are continued after delivery for a period of at least six months or longer, as indicated.

Results

There were 32 infants born alive and well to these 29 patients; 2 infants were premature as judged by their weight, one weighing 1,750 gm., the second 2,400 gm.; 3 infants weighed more than 4,000 gm.; the remaining infants were of average weight. It is recommended that BCG be given within a few days of delivery to infants born of tuberculous mothers.

The question has been asked, what effect, if any, the antimicrobial drugs have on the fetus or the newborn infant. Since the advent of antimicrobial therapy, several hundred patients have been delivered in this hospital who have received streptomycin and/or isoniazid during their pregnancies. Some patients had been treated before conception, and therapy was continued during the entire pregnancy. No injuries or untoward effects were noted at birth or after several years in all of the infants who were available for follow-up observation. This point is emphasized because the statement has occasionally been heard that streptomycin should not be used in the first trimester of pregnancy, that is, during the time of development of the fetal anlage, for fear of producing injury or anomalies in the fetus. In the dosages used today, namely, 1 gm. of streptomycin two or three times a week and 5 mg. per kg. of isoniazid daily (approximately 300 mg. daily), there appears to be no valid reason to withhold these drugs from the pregnant tuberculous patient.

TABLE 5

Results and Duration of Follow-up Study

Period of Follow-up Study	Progressed	No Change	Improved
Less than three months	1	0	0
Four to twelve months	1	3	0
More than twelve months	0	22	5
Total	2	25	5

Follow-up observation: With the exception of one patient who left the hospital on her own decision one week after delivery, all of the patients were followed for periods from five to seventy-three months (table 5). This appears to be an adequate period of follow-up to determine whether gestation will have any effect on the tuberculous process. It has been the experience of the present writers that, if gestation is to affect a pulmonary tuberculous lesion adversely, the ill effects will become manifest within three months following delivery.

Patients showing progression of disease: Two patients showed a progression of disease following delivery. Their cases are presented below.

Case 1: A 24-year-old white female was diagnosed as having moderately advanced active pulmonary tuberculosis in 1948. A left three-stage thoracoplasty was performed in 1949, following which her disease became arrested. In June, 1952, she had a difficult mid-forceps delivery of a 3,030 gm. female infant after a ten-hour labor. She was not seen by her private physician until October, 1953, when she was in her seventh month of pregnancy. She had been moving about the country and had not sought medical care during her pregnancy. Before she could be adequately studied, she delivered prematurely a 2,400-gm. male infant in November, 1953. A sputum culture following delivery was positive for tubercle bacilli but the patient had already left the hospital on her own responsibility and moved out of the state.

Case 2: A 34-year-old white female was diagnosed as having far advanced pulmonary tuberculosis in 1949. She was given streptomycin in 1949 and streptomycin and PAS in 1950. In April, 1950, she had a left upper lobectomy. She presented herself at The New York Lying-In Hospital in the third trimester of her pregnancy. Sputum examination was negative for tubercle bacilli at that time. In May, 1953, she had a low-forceps delivery of a 3,510 gm. male infant after a four-hour labor, receiving meperidine and pentobarbital for analgesia and pudendal block for anesthesia. Five months following delivery, in October, 1953, she was readmitted to The New York Hospital with active tuberculosis in her right upper lobe. She was given isoniazid, streptomycin, and PAS and kept in the hospital for three months. Following discharge she was continued on antimicrobial therapy for two years and is improved.

Patients showing no change in their condition: In 22 patients who had twenty-five deliveries there was no change in condition for at least three months following delivery. One of the patients (*Case 2*) had two deliveries and showed a progression after her second delivery. All of the other patients were followed from five months to six years after delivery and have remained well.

Patients showing improvement after delivery: Five patients with active tuberculosis during gestation were improved following delivery. Three patients were operated upon during gestation; 2 of these patients had lobectomies in the first trimester. One of them had a second uneventful delivery at another hospital following her operation, and the second had a normal full-term delivery in 1957. Both of the patients are in good condition. The third patient had a lobectomy and thoracoplasty in the second trimester. Her case is reported below:

Case 3: A 24-year-old white female was first seen in the Obstetric-Pulmonary Clinic of The New York Lying-In Hospital in February, 1953. Chest roentgenograms revealed a cavity in the right lung, and the patient was admitted to the hospital. Her past history revealed the onset of pulmonary tuberculosis in 1946, at which time she was admitted to The New

York Hospital, placed on bed rest, and given 3 gm. of streptomycin daily for ninety days. She was transferred to a sanatorium and was treated with bed rest for one year. Her original disease was on the left side, but in October, 1948, a cavity in the right upper lobe was noted. Phrenic crush, pneumothorax, and pneumoperitoneum were performed from 1948 to 1952, but her condition did not improve. Because of amenorrhea since October 27, 1952, the patient was seen in the Antepartum Clinic in January, 1953, when an intrauterine pregnancy of nine weeks' duration in a primigravida was diagnosed and the patient was transferred to the Obstetric-Pulmonary Service.

Following admission to the hospital she was given 1 gm. of streptomycin three times a week; PAS, 12 gm. daily; and isoniazid, 300 mg. per day. After two months of therapy, the cavity in the right upper lobe had not decreased in size and, after consultation with the phthisiologist and the chest surgeon, it was decided to do a right upper lobectomy in April, 1953, in the twenty-sixth week of gestation. Two weeks later, a right thoracoplasty was done. Following these operative procedures, her antepartum course was uneventful. Repeated sputum smears and gastric washings were negative for acid-fast bacilli, and her roentgenogram remained unchanged. On August 3, 1953, she had a breech extraction of a 3,400 gm. male infant. She received pentobarbital sodium and meperidine for analgesia, and pudendal block anesthesia. Her postpartum course was uneventful and she was discharged three weeks after delivery. The patient was advised to remain on modified bed rest for six weeks. Antituberculous chemotherapy was continued as follows: PAS was discontinued six months after lobectomy; streptomycin, one year after operation; isoniazid was continued for two years.

In January, 1958, she had a normal spontaneous delivery of a full-term infant after a four-hour labor. Her condition at present is good.

The fourth patient who improved following delivery had an extrapleural plombage performed three years before delivery. Her history is reported below.

Case 4: A 33-year-old white, para 0 gravida II, female was first admitted to The New York Lying-In Hospital on December 1, 1954. Pulmonary tuberculosis was first diagnosed in 1948, when she was hospitalized and treated for two years with bed rest. She continued to have sputum positive for tubercle bacilli, and progression of her disease was noted roentgenographically. In 1949, the patient became pregnant and was admitted to a tuberculosis sanatorium where a therapeutic abortion was done. The indication was bilateral, far advanced, progressive pulmonary tuberculosis. Following this she was given streptomycin, 1 gm. three times a week for five months, and pneumoperitoneum was started after an attempt at pneumothorax had failed. These procedures did not control her disease and she continued to have infectious sputum. In December, 1951, the patient was given PAS, and streptomycin therapy was resumed. In May, 1952, an extrapleural plombage was done on the left side and the pneumoperitoneum was abandoned. Sputum continued to be infectious, but by May, 1953, stabilization of lesions was noted roentgenographically; in October, 1953, sputum had been noninfectious for six months.

The patient was first seen in The New York Hospital Obstetric-Pulmonary Clinic in September, 1954, when she was fifteen weeks' pregnant. Comparison of her serial roentgenograms revealed no changes, but a sputum culture reported on October 25, 1954, was positive for tubercle bacilli. She was admitted to the hospital on the Obstetric Service, and streptomycin and PAS were continued and isoniazid, 300 mg. daily, was added. The remainder of her pregnancy was uneventful and she was delivered by low forceps of a 2,590 gm. normal infant on March 8, 1955, after eight and one-half hours' labor. Meperidine was used for analgesia and pudendal block for anesthesia.

Following delivery, the antimicrobial drugs were continued. The patient was kept on the obstetrical floor for three weeks and was then transferred to a tuberculosis sanatorium for further care. Follow-up observation in January, 1956, showed her condition to be stabilized;

sputum was noninfectious and roentgeongrams showed no change. At that time indefinite continuation of antituberculous therapy was contemplated.

The fifth patient who had active tuberculosis during pregnancy was operated upon following delivery and improved.

Case 5: A 24-year-old white female was first admitted to The New York Lying-In Hospital in January, 1955, in her twentieth week of gestation. In November, 1954, she developed pneumonia and was told that she had tuberculosis on the basis of roentgenographic findings and sputum positive for tubercle bacilli. Streptomycin, PAS, and isoniazid were begun in January, 1955. An attempt at pneumothorax in February was not successful. However, sputum was noninfectious in March, 1955, and, since she was now in her seventh month of gestation, it was decided to continue chemotherapy and re-evaluate her condition after delivery.

On May 11, 1955, she had a normal, spontaneous delivery of a male infant weighing 3,160 gm. after a three-hour labor. Caudal anesthesia was used. Chemotherapy was continued following delivery, but it was obvious on roentgenographic examination that the cavity remained open and that the pneumothorax had not been effective. Therefore, on August 16, 1955, a left upper lobe lobectomy and partial left lower lobe lobectomy were performed, followed on August 30 by a left thoracoplasty. Chemotherapy was continued, and the patient was discharged in January, 1956. In September, 1956, when she was two and one-half months' pregnant, she was again seen by the Obstetric-Pulmonary Service. Streptomycin was discontinued in November, 1956, but isoniazid and PAS were continued throughout pregnancy. Her antepartum course was uneventful, and she delivered on April 23, 1957. She was seen three months following delivery, when sputum was noninfectious and the roentgenogram showed no change in the pulmonary status.

Discussion

The question has frequently been asked whether the diagnosis of active pulmonary tuberculosis in the first trimester of pregnancy is an indication for therapeutic abortion. An analysis of 63 patients with far advanced pulmonary tuberculosis who first came under the care of one of the present writers (G. S.) in tuberculosis hospitals and who had therapeutic abortions before antimicrobial therapy was available showed that therapeutic abortion did not stop the progression of pulmonary tuberculosis. As a matter of fact, 30 per cent of the patients with far advanced disease whose pregnancies were aborted died or progressed, compared with 14 per cent in whom the pregnancy was permitted to continue to term, and active treatment for the tuberculosis was instituted (10).

The present writers reported on 66 therapeutic abortions and 34 spontaneous abortions treated at The New York Lying-In Hospital from September, 1932, through January, 1952 (11). They concluded from both clinical experience and statistical review that abortion does not improve the end results in tuberculous patients. They are so firmly convinced of this that for the past six years therapeutic abortion for pulmonary tuberculosis has not been performed on the ward service at The New York Lying-In Hospital and was done only on two occasions on the private service.

Another question that arises in connection with the pregnant patient with active tuberculosis is when surgery should be performed. Active tuberculosis does not remain stationary; it either progresses or regresses. There is no doubt that

the earlier treatment is instituted, the better are the end results. Chamberlain has stated that timing is as important to the successful surgical result as it is in a military operation, and "that neither streptomycin or any other drug can be expected to replace sound judgment in selecting the right operation for a specific patient, to be done at the proper time, under good anesthesia, by a competent surgeon" (12). The present writers concur in this viewpoint and believe that any procedure to cure tuberculosis that would be performed in the nonpregnant patient should be performed in the pregnant woman. In the 3 patients operated upon during pregnancy, two operations were performed in the first trimester and one in the second trimester. All of these patients had a course of antimicrobial therapy which did not arrest their lesions, so that surgery was deemed necessary. Holden and associates (4) report that all major thoracic operations during pregnancy among their patients were performed in the first and second trimesters.

The present writers would not advise operation in the last two months of pregnancy because of the danger of premature delivery. This danger exists for major surgery (thoracic or otherwise) performed in any pregnant patient at that time and is not a factor of the tuberculosis. Such was the rationale in the patient of *Case 5* who first came under the writers' care in the twentieth week of gestation. The present practice in the care of the pregnant patient with active pulmonary tuberculosis with cavity formation is to treat the patient with intensive antimicrobial therapy for at least six to nine months to determine whether chemotherapy will cause a regression of the lesion. If healing commences and appears to be satisfactory, chemotherapy is continued for a longer period. If regression of the lesion does not occur after a reasonable period of therapy, and if the lesion is one that is amenable to it, surgery is performed.

While major thoracic surgery may be safely performed during pregnancy, it is believed that it is best undertaken in the nonpregnant state and should only be performed during pregnancy under exceptional circumstances. On the other hand, there seems to be no reason to interdict pregnancy in women who have had major thoracic operations.

Clinically none of the patients in this study experienced dyspnea or respiratory embarrassment during the latter months of pregnancy or during labor. This may be due to the fact that the patient's activity was limited in the last few months of pregnancy and also to the use of oxygen at the time of delivery. Gaensler and associates (13) studied the effect of pregnancy in patients with moderate pulmonary insufficiency as a result of collapse procedures. During the ninth month of pregnancy, the observed changes were similar to those in normal subjects. A 35 per cent increase in resting ventilation was achieved by increasing the volume of tidal excursions. Unlike the condition in normal subjects, however, minute ventilation increased only enough to supply the augmented oxygen demand, and the ventilatory equivalent was not larger than before pregnancy. In the series of Gaensler and associates, patients who had bilateral surgery showed no alarming signs referable to the respiratory or circulatory systems during labor. An unexpected finding was the absence of dyspnea in these patients during pregnancy.

Murdock (6) noted dyspnea in only one of his 15 patients who had major

thoracic operations before pregnancy, and this was considered to be functional rather than organic.

Corner and Nesbitt (5), from a review of 42 cases in the literature plus their own 15 cases, conclude that pneumonectomy and pulmonary resection are not contraindications to pregnancy provided the patient is not dyspneic at rest or on slight exertion before conception.

Birath and co-workers (14) studied 51 patients who underwent resection or lobectomy for preoperative and postoperative unilateral functional loss. Before resection, the diseased lung had already lost from one-tenth to one-third of its normal function, depending on the extent and site of the disease. (In patients with lower lobe involvement, one-half of the function was lost before surgery.) Three months after operation these losses were increased and ranged from one-fourth to two-thirds of the normal function. The poorer function following the larger resections is explained by the increase in large exudate formation, bleeding, and the hindrance to expansion of the remaining lung regions due to their position changes. The fact that the lower lobectomy group had a larger functional loss is believed to be attributable to the heightened tendency for the diaphragm to be drawn up and immobilized. From these studies, Birath and associates state that all signs point to the likelihood of functional improvement after the three-month postoperative period. This would be further reason for not performing major thoracic surgery in the last trimester of pregnancy, since the loss of function of the lung would then be greatest three months after surgery—at the approximate time of delivery.

Birath and Söderholm (15) also studied bronchospirometrically 22 patients undergoing thoracoplasty (four to six ribs) whose opposite lungs were apparently healthy. Preoperatively the diseased lung had already lost 24 to 30 per cent of its oxygen uptake and vital capacity. After surgery the loss was increased from the calculated normal level for oxygen uptake to 39 per cent in the right-sided group and 47 per cent in the left-sided group; for vital capacity the figures were 41 and 40 per cent, respectively. After postoperative stabilization of pulmonary function, they estimated that the total functional loss for the diseased and operated lung would be about one-third of its normal value, calculated for the patient in the supine position.

Summary

Eleven per cent of 257 tuberculous patients delivered at The New York Lying-In Hospital between 1952 and 1956 had major thoracic operations for pulmonary tuberculosis performed before or during pregnancy. Twenty-five operative procedures were performed before conception, three during pregnancy, and one shortly after delivery.

Labor was managed with small repeated doses of Demerol® for analgesia, and local or pudendal block was the anesthesia of choice for delivery. Low-forceps delivery was performed to shorten the second stage of labor if this was prolonged or tiring. A longer period of postpartum rest was advised, and breast feeding was not permitted.

The results following the thirty-two deliveries in 29 patients showed no change in the disease in 25 instances, improvement in 5 patients in whom the disease was active during pregnancy, and progression of the disease in 2 instances. Follow-up observation was from five to seventy-three months after delivery. These results compare favorably with those in nonpregnant tuberculous patients with similar disease and show that pregnancy may safely be undertaken following major thoracic surgery. While major thoracic surgery may be carried out during the first and second trimesters of pregnancy (it should not be done during the third trimester because of the danger of premature labor), the present writers believe it is best performed during the nonpregnant state.

Sumario

Parto a Término a Continuación de Cirugía Torácica Mayor por Tuberculosis

En 11 por ciento de 257 tuberculosas que dieron a luz en el Hospital de Maternidad de Nueva York entre 1952 y 1956 se ejecutaron operaciones torácicas de cirugía mayor por tuberculosis pulmonar antes del embarazo o durante éste. Veinticinco procedimientos quirúrgicos se llevaron a cabo durante la concepción, tres durante la gestación y uno poco después del alumbramiento.

El parto fué atendido con pequeñas dosis repetidas de Demerol® para analgesia y el bloqueo local o pudendo fué la anestesia de elección para la extracción. Para acortar el segundo período del parto, si éste era prolongado o fatigoso, se ejecutó la extracción con fórceps bajo. Se aconsejó un período más largo de descanso postpuerperal, vedándose la amamantación.

Los resultados consecutivos a los treinta y dos partos en 29 enfermas no revelaron alteración de la dolencia en 25 ocasiones, y mostraron mejoría en 5 pacientes en las que la enfermedad había sido activa durante el embarazo y agravación del mal en 2 casos. La observación subsiguiente duró de cinco a setenta y tres meses después del parto. Estos resultados se comparan favorablemente con los observados en tuberculosas no gestantes cuya enfermedad era semejante y demuestran que puede abordarse inocuamente el embarazo después de la cirugía torácica mayor. Aunque ésta puede ejecutarse durante el primero y el segundo trimestres de la gestación (no debe ejecutarse durante el tercer trimestre debido al riesgo de un parto prematuro), opinan los AA. que es mejor llevarla a cabo cuando no existe embarazo.

Resume

Accouchement à terme après chirurgie thoracique majeure pour tuberculose

Onze pour cent des 257 tuberculeuses ayant accouché au New York Lying-In Hospital, entre 1952 et 1956, avaient été soumises à des interventions chirurgicales thoraciques majeures pour tuberculose pulmonaire avant ou au cours de la grossesse. Vingt-cinq procédés chirurgicaux ont été pratiqués avant la conception; trois durant la grossesse et un aussitôt après l'accouchement.

De petites doses répétées de Démerol ont été administrées comme analgésique pendant la travail; pour l'accouchement, on a eu recours au blocage local ou à celui du nerf honteux interne comme thérapeutique anesthésique de choix. L'application de forceps bas a été effectuée pour raccourcir le deuxième temps du travail si celui-ci se prolongeait ou fatiguait la malade. Une période prolongée du repos post-partum était recommandée et l'allaitement au sein interdit.

Parmi 29 malades, les résultats succédant à trente-deux accouchements n'ont montré aucune modification de la maladie dans 25 cas; une amélioration a été observée chez 5 malades ayant une tuberculose en activité durant la grossesse et, dans 2 cas, la maladie a progressé. La période d'observation après l'accouchement a été de cinq à soixante-treize

mois. Ces résultats soutiennent la comparaison avec ceux obtenus dans des cas de tuberculose comparable chez des tuberculeuses non enceintes et ils prouvent qu'une gestation peut être entreprise sans danger après une intervention chirurgicale thoracique majeure. Bien que la chirurgie thoracique majeure puisse être pratiquée durant le premier et le deuxième trimestre de la grossesse (elle doit être exclue durant le troisième trimestre en raison du risque de travail prématuré), les auteurs estiment préférable de la pratiquer en l'absence de l'état de grossesse.

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THE EFFECT OF GLUCOSE ON THE TUBERCULIN REACTION IN TISSUE CULTURE[1,2]

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INTRODUCTION

The classic tissue culture investigations of Rich and Lewis (1, 2) clearly demonstrated the existence of cellular sensitivity in tissues from animals which were hypersensitive to tuberculin. Both Aronson (3) and Moen (4, 5) indicated the specificity of this sensitivity. Since the *in vitro* sensitivity was not dependent upon circulating antibody, but had a specificity analogous to antibody systems, the hypothesis arose that this cellular sensitivity might be due to a sort of sessile antibody firmly attached to the cells and resulting in cellular injury when exposed to the antigen.

However, measurements of the respiration (6–8) or glycolysis (8) of sensitive tissues in the presence or absence of tuberculin had failed to demonstrate any differences between the two. Since it was to be expected that cell death should result in a reduction of respiration or glycolysis, the failure to observe these effects led some workers to question the validity of cellular sensitivity (8).

Some preliminary studies with splenic explants in the Warburg apparatus indicated to the present writers that there was a reduction in respiration of sensitive cells in the presence of purified protein derivative (PPD), but only after thirty hours' contact with the antigen. Since the technique suffered from several disadvantages (inability to observe the cells microscopically, difficulty in maintaining sterility, occurrence of a mixed cell population), it was decided to measure the glucose utilization of monocyte cultures instead.

Although monocyte cultures in Porter flasks abolished the difficulties encountered with the Warburg, it became evident that the glycolysis of sensitive cells was not affected by PPD. This apparent discrepancy with the expected results was resolved when it was noticed that glucose could, in fact, delay the appearance of the cellular injury imposed upon sensitive cells by PPD. It was also observed that sensitive monocytes, if maintained in tissue culture, could lose completely the ability to react to PPD.

MATERIALS AND METHODS

Hanks solution: Hanks solution, ten times concentrated, was prepared according to the directions given by J. H. Hanks (9) except that the glucose was omitted. The solution was sterilized with a Selas filter and diluted with sterile distilled water just prior to use. Glucose, as a sterile 10 per cent solution in water, was added to the solution as required in the individual experiments.

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[2] This research has been aided by Grant No. E–467 (C3) from the National Institutes of Health, Public Health Service.

Bicarbonate solution: A 1.4 per cent sodium bicarbonate solution containing 0.002 per cent phenol red was sterilized by autoclaving at 10 pounds of pressure for ten minutes. This solution was used to adjust the pH of other solutions. When used in preparing the medium for tissue culture, it was saturated with carbon dioxide by bubbling the gas through the solution with a sterile pipette immediately before use. This latter solution had a pH of 7. It will subsequently be referred to as "gassed bicarbonated solution."

Penicillin-streptomycin-nystatin solution: A stock solution of penicillin, streptomycin, and nystatin was aseptically prepared in saline solution to give a concentration of 5,000 units of penicillin, 5,000 γ of streptomycin, and 3,000 units of nystatin per ml. Final concentrations in the medium were 50 units, 50 γ, and 30 units, respectively.

PPD solution: PPD solution was a stock solution containing 1.5 mg. of PPD[3] per ml. of 30 per cent guinea pig serum in Hanks solution. The preparation was kept frozen until used. There was no detectable loss of activity over one year when the solution was stored in this manner.

Either 0.025 ml. or 0.05 ml. of this solution was added as required to flasks containing 1.5 ml. of medium, thus giving final concentrations of 25 or 50 γ per ml., respectively.

Glucose determination: The method of Folin and Malmros (10) was used on samples freed of protein by the $Ba(OH)_2$—$ZnSO_4$ precipitation procedure of Somogyi (11). A 0.05-ml. sample was sufficient for triplicate determinations. For the sake of convenience, samples were collected and frozen until the end of the experiment, when all of the determinations were carried out at once.

Vaccination of guinea pigs: All guinea pigs used in these studies were of the Hartley strain.[4] Animals were vaccinated with 5 mg. of living BCG (Phipps) inoculated subcutaneously in the nape of the neck. This route was chosen because earlier observations had revealed that guinea pigs vaccinated by the intraperitoneal route yielded fewer exudate cells when subsequently stimulated for cell collection.

The animals were skin tested with 0.5 γ of PPD four weeks after vaccination. Reactions usually consisted of induration 15 to 17 mm. in diameter, with a central necrosis 5 to 7 mm. in diameter. Most animals were used within three weeks after being skin tested.

Tissue culture procedure: Guinea pigs were given 10 ml. of mineral oil intraperitoneally forty-eight hours before the tissue cultures were prepared. Originally exudates were stimulated with glycogen according to the method described by Suter (12), but this technique was discarded when it was found to provide no more monocytes than were normally present in the peritoneal cavity.

At the time of culture, Hanks solution, concentrated ten times, was diluted with distilled water and adjusted to pH 7.4 with bicarbonate solution. An additional milliliter of gassed bicarbonate was then added for every 200 ml. of Hanks solution prepared in order to increase the buffering capacity. This did not alter the pH immediately, but, on standing, the pH would gradually increase to 8.2; however, the solution was used before an appreciable increase in pH occurred. The guinea pigs were killed by a blow on the head and the peritoneal cavity was opened aseptically. Each cavity was washed out with 100 ml. of Hanks solution, containing heparin[5] 1:20,000 and 1 per cent guinea pig serum. The washings were centrifuged for three minutes at 250 g., the supernate and oil were poured off, and the cells were gently resuspended in 10 ml. of Hanks solution containing 4 per cent guinea pig serum. The cells were again centrifuged in the same manner and resuspended in 10 ml. of the same fluid.

One drop of the suspension was placed in a hemocytometer and counted, while 0.5 ml. was centrifuged in preparation for making differential smears. These smears were prepared in the following manner:

After the 0.5-ml. portion was centrifuged, the supernate was poured off and the cells were suspended in 0.3 ml. of normal guinea pig serum and left to stand

[3] All of the PPD used in these studies was from lot 3, No. 9587, which was generously supplied through the courtesy of Merck Sharp & Dohme, West Point, Pennsylvania.

[4] An inbred albino strain supplied by John C. Landis, Hagerstown, Maryland.

[5] Manufactured as Liquaemin Sodium by Organon, Inc., Orange, New Jersey.

for five minutes. Smears were then made in the same manner employed for blood. After rapid air drying, the smears were stained with Wright's stain and examined in the customary way.

On the basis of the total leukocyte count and the differential, the volume of cell suspension providing 3,000,000 monocytes was calculated. If this was less than 1 ml., an additional 1 ml. of Hanks solution with 4 per cent guinea pig serum was added to each Porter flask prior to adding the cells; otherwise the cell suspension was introduced into the flasks directly. The flasks were immediately shaken so that the entire floor was evenly covered with the suspension. The flasks were stoppered and placed in an incubator for twenty minutes in order to allow the cells to settle onto the glass. During this interval the medium was prepared.

Previous work had shown that the cells require a small amount of glucose (30 mg. per 100 ml.) in order to survive. Furthermore, a high bicarbonate concentration (approximately 2 gm. per liter, the same as found in plasma) was decidedly beneficial. The minimal serum concentration for survival was found to be 5 to 10 per cent. On the basis of these observations, the following medium was employed: 20 per cent of normal guinea pig serum, 15 per cent of gassed bicarbonate solution, 65 per cent of Hanks solution (containing the antimicrobials and sufficient glucose to provide either a minimal final concentration of 30 mg. per 100 ml. or more as required in the individual experiments).

After the cells had settled for twenty minutes, the flasks were shaken to dislodge nonadhering cells, and the fluid was poured off. Immediately, 1.5 ml. of the medium were added, the flasks were restoppered and placed in the incubator. The cultures were examined at suitable intervals and usually photomicrographed in order to obtain a permanent record for comparison at the end of the experiment.

The medium was never changed, although bicarbonate solution was added as needed to maintain the pH between 7.0 and 7.6. The pH was determined by a direct comparison with LaMotte phenol red pH standards.

Results

Morphology of monocytes in tissue culture: Monocytes in tissue culture were observed to exhibit a regular pattern of morphologic changes when viewed with the light microscope at 100 to 400 magnifications. For the first few days, the cells were rather round or elongated with one or two pseudopods. The cytoplasm contained a few vacuoles and thus appeared slightly granular. These vacuoles are not to be confused with ingested mineral oil which appeared as highly refractile droplets in the cytoplasm. Approximately on the third to the sixth days, the cells suddenly flattened out, showing prominent membranes or several long thin pseudopods with small visible membranes at the distal ends. The cytoplasm was very transparent, but sometimes contained a few vacuoles, especially near the periphery of the cell.

The exposure of monocytes from a tuberculin-sensitive animal to PPD brought about the following changes: At about twenty-four to thirty-six hours after contact with PPD the cells were found to be round, and the cytoplasm was decidedly more vacuolated than the controls. There was a marked decrease in the phagocytic activity of the cells as evidenced by the failure to phagocytize other leukocytes (neutrophils, lymphocytes, and eosinophils) still remaining in the culture. During the next few days, the vacuoles in the cytoplasm coalesced into one or more large vacuoles, the nucleus being compressed along the side of the cell. At this stage, the cells spontaneously floated off the surface of the flasks, and it was difficult to find an area with even a few cells sticking.

Normal cells showed no reaction when exposed to PPD concentrations as high as 100 γ per ml.

The above reactions are in agreement with those already reported by Rich and Lewis (2) and Aronson (3) who mentioned that vacuolation of sensitive monocytes occurs in the presence of OT; but no detailed description of the process was given.

Minimal concentration of PPD necessary: It was found that as little as 1 γ of PPD per ml. was sufficient to produce an effect in sensitive monocytes, although the reaction was not maximal. At these concentrations of PPD, the cells showed increased vacuolation of the cytoplasm and a greater tendency to round up as compared with controls. As the concentration of PPD was raised, the vacuolation was increased until at a concentration of 5 γ per ml. the maximal effect could be obtained. At this concentration, however, the reaction does require longer for its appearance than at concentrations of 10 to 50 γ per ml.

Stability of PPD in tissue culture: In order to ascertain the persistence of biologically active PPD in monocyte culture, a number of flasks of normal or sensitive cells mixed with PPD (50 γ per ml.) were prepared, and 0.1-ml. aliquots of the cell-free medium were periodically inoculated intracutaneously into sensitive guinea pigs. There was no detectable loss of activity over a thirty-week period of incubation when compared with that produced by the similar inoculation of a freshly prepared PPD solution containing 5 γ per 0.1 ml. dose. Medium from control flasks without PPD did not elicit any skin reactions in these guinea pigs.

Glucose utilization in the presence of PPD: Two series of flasks were prepared, one containing monocytes from a normal guinea pig, and the other cells from a sensitive animal. The glucose concentration of the medium was approximately 250 mg. per 100 ml. (3.75 mg. per flask). About twenty-four hours later, PPD was added to half the flasks of each series to give a final concentration of 50 γ per ml. Samples of the medium were removed at intervals for glucose estimations. Care was taken not to allow the pH of the medium to drop below 7.0 since previous experience had demonstrated that, if the pH of the culture decreased to this level, glucose utilization was markedly decreased, and if a pH of 6.8 or below was maintained for any length of time, the cells were injured. Furthermore, it was imperative that any fluid that might have run into, or condensed on, the neck of the flask be thoroughly mixed with the medium before the samples were taken out; otherwise, serious errors would be introduced into the determinations. Suitable corrections were made for the removal of samples and the dilution with bicarbonate solution when determining the glucose consumption.

The rate of glucose consumption was essentially constant during each individual experiment, but depended on the original amount of glucose supplied; the greater the initial glucose concentration, the higher the rate of utilization. The rates usually fell within the range of 0.1 to 0.3 mg. per million cells per twenty-four hours.

There was no difference in the glucose utilization of sensitive monocytes in the presence or absence of PPD. The same was true of normal cells. It should be emphasized that it was not possible to compare quantitative data from one

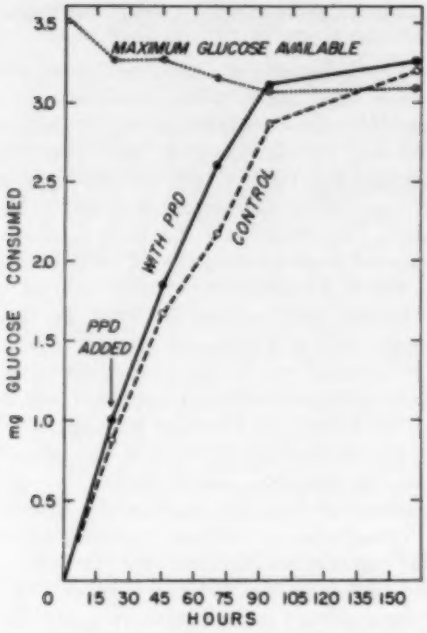

FIG. 1. Glucose utilization of sensitive monocytes in the presence and absence of PPD.

guinea pig with such data from another. There was sufficient variation between the cells from different guinea pigs to necessitate using the cells from only one animal under the particular test conditions.

The glucose consumption of sensitive monocytes in the presence and absence of 50 γ of PPD is illustrated in figure 1. The rates of utilization are essentially constant until the glucose is depleted from the medium. In this experiment the extensive vacuolization indicative of the allergic reaction did not occur until two days after the glucose was gone. The dotted line curve indicates the amount of glucose theoretically remaining in the flasks, assuming no utilization, but corrected for the samples removed and dilution resulting from the addition of bicarbonate solution.

The delay of the reaction by glucose: As previously mentioned, the injury by PPD to sensitive monocytes usually became evident after twenty-four to thirty-six hours. However, in the experiments in which glucose utilization was being measured, the reaction was usually found to occur a few days later than would be expected. The only difference between these experiments was in the concentration of glucose; the earlier experiments in which the reaction was studied were performed with medium containing 50 to 100 mg. of glucose per 100 ml., whereas concentrations of about 250 to 350 mg. per 100 ml. were used in the utilization experiments. For this reason, it was decided to study the reaction in the presence of high and low glucose concentrations.

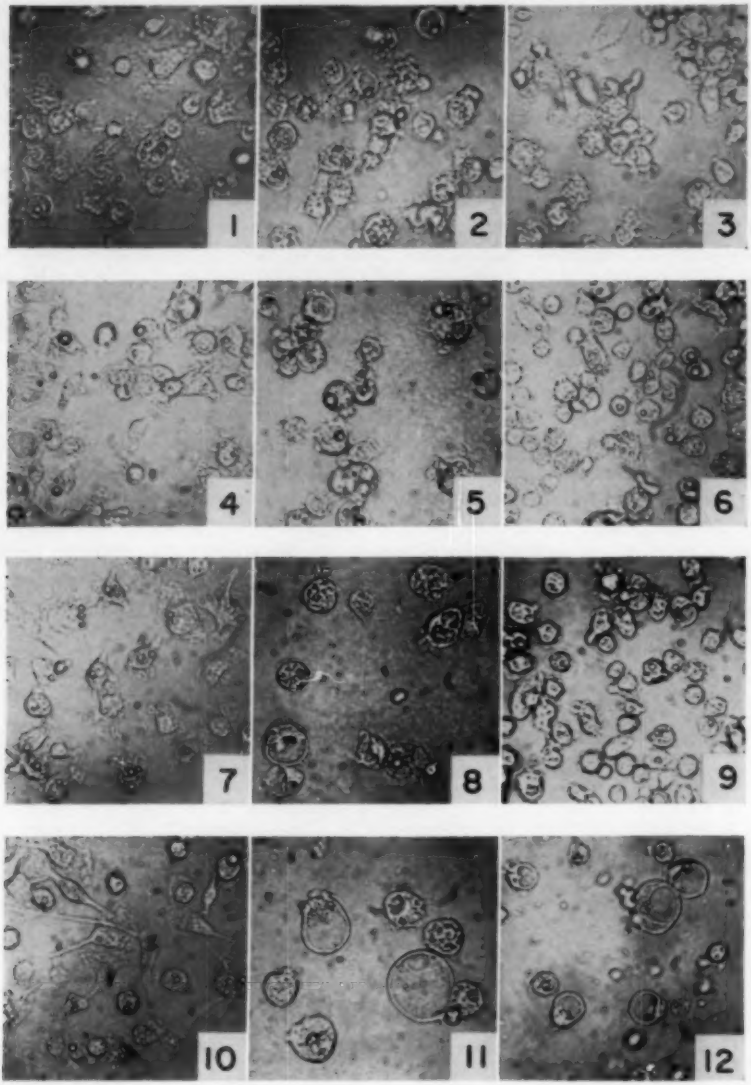

FIG. 2. Ability of glucose to delay reaction. From left to right: Control (no PPD): low glucose concentration with PPD, high glucose concentration with PPD. Top row (1-3): One day after the addition of PPD. Note beginning of vacuolation in low-glucose flask. Second row (4-6): Three days after PPD. Extensive vacuolation in low-glucose, but no effect in high-glucose flask. Third row (7-9): Four days after PPD. Large vacuoles in low-glucose and beginning of vacuolation in high-glucose flask. Bottom row (10-12): Eight days after PPD. Extensive vacuolation in both low and high glucose concentration.

Flasks containing cells from a sensitive guinea pig were divided into two series, one supplied with a low concentration of glucose (30 to 50 mg. per 100 ml.) and the other with a high concentration (250 to 400 mg. per 100 ml.). PPD was added to some flasks in each series; those without PPD served as controls. The PPD was added either immediately after preparing the cultures or after twenty-four hours' incubation.

In the instances in which the PPD was added after twenty-four hours' incubation, there was a decided delay in the appearance of the tuberculin reaction in flasks containing the higher concentration of glucose. This delay varied considerably in its duration, lasting from two to ten days in different experiments. The glucose under these conditions did not abolish the reaction, since the cells were eventually affected to the same extent as in control flasks. The ability of glucose to delay the onset of the reaction is illustrated in figure 2.

When the PPD was added immediately at the start of an experiment, the results were unpredictable. Sometimes the glucose delayed the reaction, while at other times there was no detectable effect. The length of the delay in different experiments in which PPD was added after various intervals up to one day may be seen in table 1.

Loss of cellular reactivity: Due to the variability in results obtained when PPD was added immediately to cultures in the presence of high glucose concentrations, it was decided to observe further the effect of adding PPD after different periods of incubation. Two series of flasks containing cells from a sensitive guinea pig were prepared. The concentration of glucose in one series was about 30 mg. per 100 ml., and in the other, 300 mg. per 100 ml. PPD (final concentration, 25 γ per

TABLE 1

DELAY OF THE REACTION BY GLUCOSE

Time PPD Added	Low Glucose Concentration	High Glucose Concentration	Delay Period*
	mg. per 100 ml.	*mg. per 100 ml.*	*days*
Immediately	50	200 and 400	None
Immediately	50	200 and 400	None
Immediately	30	300	3
Immediately	30	300	10
2 hours	30	300	2
5 hours	30	300	2
5 hours	30	600	8
6 hours	50	300	3
1 day	50	250	2
1 day	30	300	3
1 day	30	300	9
1 day	30	300	4
1 day	50	400	3
1 day	60	400	4

* This is the difference between the time the reaction was evident in the flasks containing the low glucose concentrations and the time it occurred in presence of the higher glucose concentrations.

ml.) was added to successive flasks in each series after various intervals, for as long as seven days.

In the presence of low glucose concentrations, the flasks which had received PPD immediately always exhibited the reaction twenty-four to thirty-six hours

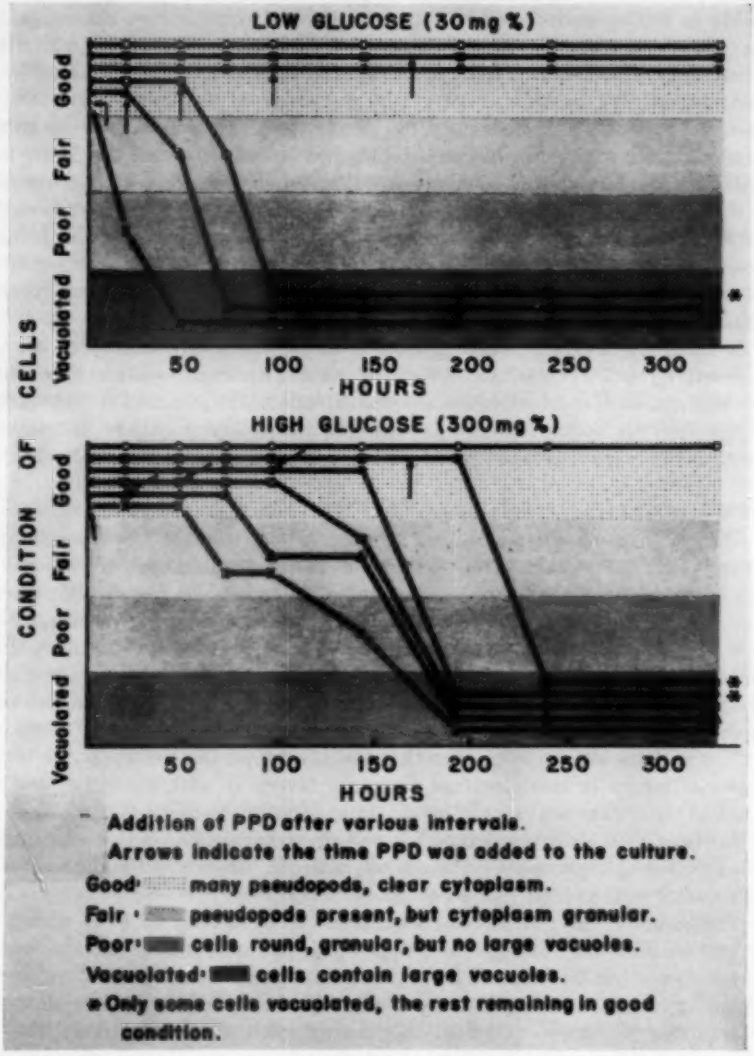

FIG. 3. Loss of cellular reactivity to PPD in the presence of low and high glucose concentrations.

later. In those flasks which had received the PPD one or more days after the start of an experiment, the results were more irregular. The first observation which became apparent was that the cells could lose their ability to respond to PPD. This loss usually occurred between the second and sixth days in tissue culture, but did occur as early as twenty-four hours in one instance. The variability in timing seems to be the result of individual differences among guinea pigs, since the experimental conditions were held as constant as possible. In those experiments in which sensitivity was lost after several days in culture, it was evident that this change was not abrupt but occurred gradually over a one- to three-day period. The addition of PPD during the first few days gave rise to the usual reaction, with extensive vacuolation and loss of cells from the flask's surface. The addition of PPD to subsequent flasks then seemed to affect fewer cells in the culture, as evidenced by an increased number of cells which survived in the flasks. In some cases, this increased survival was more prominent in the next flask in the series to receive PPD; but, in other cases, there was little reaction at all to the incorporation of PPD into the medium. Beyond this time, the cells remained completely resistant to PPD.

In those flasks in which high glucose concentrations were employed, the loss of reactivity to PPD was also evident. However, the length of time over which the cells remained susceptible to PPD was extended. The results of an experiment of this type are shown in figure 3. Note that the ability of glucose to delay the onset of the reaction is also apparent.

Discussion

The present studies again confirm the observation (which has not gone unchallenged) that macrophages from tuberculin-sensitive guinea pigs are injured by the presence of the antigen in tissue culture. They also demonstrate that glucose utilization is not disturbed when sensitive macrophages are brought into contact with PPD; in fact, the presence of a high concentration of glucose in the medium actually delays the onset of the cellular reaction. Furthermore, sensitive macrophages will eventually lose the ability to react to PPD if they are maintained in tissue culture prior to exposure to the antigen.

It is perhaps well to emphasize that the amount of PPD necessary to obtain maximal injury in tissue culture compares favorably with the concentrations used to elicit skin reactions. The maximal reaction in tissue culture could be obtained with a concentration of 5 γ per ml. (equivalent to a skin-test dose of 0.5 γ) while an observable, although not maximal, effect could still be obtained in cultures with as little as 1 γ per ml. (skin-test dose of 0.1 γ).

Furthermore, the differences between the concentration of PPD which will injure sensitive macrophages and that which has no effect on normal macrophages are a hundred fold at least. Sensitive cells, as mentioned before, are injured by a concentration of 1 γ per ml., whereas normal cells have shown no effect in the presence of 100 γ per ml. (the highest concentration tried). This is a far greater differential than that reported by Fabrizio (14), who, working with neutrophils, observed that the concentrations of PPD which would produce a

nonspecific injury to the cells was only about twice that giving a specific effect. In the majority of the present experiments, a concentration of 25 γ per ml. was used merely to conserve the supply of PPD. This is five times the amount necessary to produce a maximal reaction with sensitive cells.

While these investigations were in progress, Marks and James (8) published their findings on this problem. They elicited exudates by the intraperitoneal injection of OT and measured the glycolysis and respiration of the cells thus derived without exposure to any additional tuberculin. Respiration studies in the presence of OT, using neutrophils and macrophages obtained by the intraperitoneal injection of casein or mineral oil, were also carried out. Since they did not detect any difference in the rates of respiration and glycolysis between normal and sensitive cells, they questioned the validity of the concept that the antigen has a lethal effect on sensitive cells.

One must note, however, that Marks and James studied glycolysis of neutrophils rather than monocytes. Respiration studies were done on both mononuclear cells and neutrophils. The statement of Marks and James that no effect of tuberculin on respiration can be noted in the case of polymorphonuclears induced by OT needs further study. If one examines their data, it may be seen that in every instance the change in respiratory rate after storage of the cells at 4°C. was greater for sensitive cells than it was for normal cells. The effect is one in which sensitive cells are less active metabolically after storage. These differences, though small, seem to indicate that there was either an intrinsic difference between normal cells and sensitive cells or that there was injury of sensitive cells due to the tuberculin used as a stimulant. Final judgment must await further experimentation.

The conclusions from the present studies on glucose utilization by macrophages agree with those of Marks and James, but disagree completely with their idea that sensitive cells are not affected by tuberculin. On the basis of the present findings, it would appear that the period over which Marks and James made their measurements was insufficient for the reaction to occur. Furthermore, the subsequent observation of the present writers that glucose can delay the onset of the reaction seems to provide an additional explanation for the inability to notice an effect upon glycolysis. The means by which glucose achieves this effect is still in the realm of speculation.

It should be noted that glycolysis of sensitive cells in the presence of PPD was observed in this study for a period of only seven days. In most instances the glucose supplied to the cells was completely metabolized by the fifth day. In only 2 instances did any glucose remain in the medium at the time the cellular reaction became evident. In these 2 cases, there was some late reduction in the rate of glucose disappearance. However, the interpretation of this reduction would be difficult for the following reasons: (*a*) those cells, dislodged as a consequence of injury by PPD, would be removed from the flasks with the samples, thus resulting in an apparent decrease in glycolysis even though the cells might still be actively metabolizing the substrate; (*b*) there is some indication that macrophages will spontaneously reduce their glucose consumption when kept in

tissue culture for several days, thus bringing up the question whether a reduction in glycolysis is due to the effects of PPD or some other phenomenon. No attempt has been made to further investigate this problem of a possible delayed effect upon glycolysis by the antigen.

In addition to disagreeing with Marks and James, results of the present study are not in agreement with those of Moen (13). Moen claimed that the sensitivity of fibroblasts, supposedly derived from mononuclear cells, persisted through a number of generations, whereas the present writers found that the ability to respond to PPD is lost rather readily after culturing. These experimental results indicate that the loss of reactivity to PPD in the case of macrophages occurs after various periods in tissue culture. In some cases the cells will no longer be damaged by PPD after twenty-four hours in Porter flasks, whereas others have maintained susceptibility to the antigen for as long as six days. This loss of reactivity to the PPD does not occur simultaneously in all of the cells of a single culture; some cells retain their susceptibility as much as two days longer than others in the flasks. Furthermore, the presence of high concentrations of glucose in a culture will extend the period over which the cells will react to PPD.

To account for the discrepancy between the present observations and those of Moen, an explanation is offered. Judging from the photographs and descriptions provided by Moen, in the present writers' opinion, the cells involved were actually fibroblasts and not macrophages that had "transformed into morphologically typical fibroblasts." Some monocytic cultures have occasionally been contaminated with fibroblasts (possibly introduced as small bits of mesentery torn loose while harvesting the exudate) which developed into small colonies, but these appeared to be rather resistant to PPD (up to 100 γ per ml.) and were not studied in detail. Furthermore, even though the monocytes can be maintained for considerable periods of time, no multiplication of these cells has been observed in tissue culture. The apparently high frequency of proliferation of fibroblasts in Moen's cultures may have been due to his use of plasma and tissue and embryo extracts, whereas the technique of the present writers, of having the cells adhere directly to glass in the absence of tissue extracts, provides rather poor growth conditions for isolated fibroblasts. Another difference between the present experimental conditions and those of Moen was that, in the present instance, the medium was not changed during an experiment; whereas Moen did change the medium. However, it is difficult to say whether or not this procedure in itself could actually account for any differences in the observed results.

Whether the mechanism of the tuberculin skin reaction can be explained in terms of the *in vitro* findings is difficult to say. Since monocytes are highly sensitive to PPD (as compared with fibroblasts) it might be that they are involved in initiating the skin reaction. Even though the reaction in tissue culture with low glucose concentrations requires about twenty-four to forty-eight hours to occur, it may be that in the skin the cellular injury becomes evident sooner, perhaps by the early release from injured leukocytes of some toxic agent capable of producing the inflammation found in the skin lesion.

Summary

Monocytes obtained from peritoneal exudates of normal and tuberculin-positive guinea pigs were maintained in tissue culture and studied in the presence of PPD. Sensitive cells on exposure to PPD showed progressive vacuolation of the cytoplasm, while normal cells were not affected by the same concentration of the antigen. There is at least a hundred fold difference in the concentration of PPD which will injure sensitive macrophages and still not affect normal cells.

Glucose utilization of sensitive cells is not affected by PPD. Glucose, however, has the ability to delay the appearance of the *in vitro* tuberculin reaction, but cannot inhibit it completely. This delay lasted from two to ten days, depending on the individual guinea pig.

The sensitive monocytes after being maintained in tissue culture lost the ability to react to PPD. The period of sensitivity lasted from one to six days, depending on the particular guinea pig; but was somewhat extended in the presence of high glucose concentrations. The loss of sensitivity in a culture was not uniform, but occurred in some cells before others.

Sumario

El Efecto de la Glucosa sobre la Reacción a la Tuberculina en el Cultivo de Tejidos

Monocitos obtenidos de exudados peritoneales de cobayos normales y tuberculinopositivos fueron mantenidos en cultivos de tejidos y estudiados en presencia de PPD (DPP). Las células sensibles al ser expuestas al PPD revelan vacuolación progresiva del citoplasma en tanto que no afecta a las normales la misma concentración de antígeno. Hay a lo menos un céntuplo de diferencia en la concentración de PPD que lesionará los macrófagos sensibles y, sin embargo, no afectará los normales.

La asimilación de glucosa por los glóbulos sensibles no se ve afectada por el PPD. No obstante, la glucosa posee la facultad de demorar la aparición de la reacción a la tuberculina *in vitro*, pero sin poder inhibirla del todo. Esta demora dura de dos a diez días, conforme al cobayo dado.

Los monocitos sensibles, después de ser mantenidos en cultivos de tejido, pierden la capacidad para reaccionar al PPD. El período de sensibilidad dura de uno a seis días, conforme al cobayo dado; pero se alarga algo cuando existen altas concentraciones de glucosa. La pérdida de sensibilidad en un cultivo no es uniforme, ocurriendo en algunos glóbulos antes que en otros.

Resume

L'effet du glucose sur la réaction à la tuberculine dans la culture des tissus

Les monocytes obtenus à partir d'exsudats péritonéaux de cobayes normaux ou dont la réaction à la tuberculine est positive ont été maintenus dans la culture de tissus et étudiés en présence de PPD. Les cellules sensibles à l'exposition au PPD montrent une vacuolisation du cytoplasme, tandis que les cellules normales ne sont pas affectées par la même concentration de l'antigène. Il existe un écart au moins cent fois plus grand entre les concentrations de PPD qui altéreront les macrophages sensibles sans modifier les cellules normales.

L'utilisation du glucose des cellules sensibles n'est pas affectée par PPD. Le glucose a toute fois le pouvoir de retarder l'apparition de la réaction à la tuberculine *in vitro*, sans l'inhiber complètement. Selon le cobaye individuel, ce délai varie entre deux à dix jours.

Les monocytes sensibles, après avoir été maintenus dans la culture, perdent la capacité de réagir au PPD. La période de sensibilité dure de un à six jours, selon le cobaye particu-

lier; elle peut s'allonger un peu en présence de concentrations élevées de glucose. La perte de sensibilité dans une culture n'est pas uniforme; elle peut se produire dans certaines cellules avant d'autres.

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OBSERVATIONS ON AMBULATORY TUBERCULOUS PATIENTS WITH PULMONARY CAVITIES AND NONINFECTIOUS SPUTUM (THE "OPEN-NEGATIVE" SYNDROME)[1]

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INTRODUCTION

Since isoniazid came into widespread use in the treatment of pulmonary tuberculosis, a not infrequent outcome of the drug treatment has been a state characterized by roentgenographic stability, the absence of tubercle bacilli in the sputum, and the presence of residual air spaces in the lung which have been variously interpreted as cavities, cysts, bullae, and similar entities. The problems of the prognosis of such cases—for example, when to consider the disease active or inactive and when to advise resectional surgery—have been widely debated. Most of the arguments brought into such discussions were based on pathologic findings in specimens obtained by resection or at autopsy (1–5). Reports of clinical follow-up observations in this type of case are scarce in the American literature. One such report by Bell, Decker, and Raleigh (6) deals with follow-up observations after only short-term drug therapy and in patients whose sputum had been free of tubercle bacilli for only three months. One paper by Corpe and Blalock (7) deals with patients who had long periods of stability and noninfectiousness during, and subsequent to, hospital treatment for tuberculosis.

The present paper is a report of the results of clinical follow-up study in a group of 94 patients whose disease had become noninfectious and roentgenographically stable under drug treatment, and who presented residual intrapulmonary air spaces roentgenographically. All patients were ambulatory for all, or a major portion, of their supervision and treatment, and were carrying out their normal day-to-day activities to the extent that their general health permitted while they were under observation. In no patient were the air spaces resected during the period of observation, either because surgery was not thought to be medically indicated or because the patient refused to undergo surgery.

MATERIAL AND METHODS

Selection of cases: The 94 patients were selected consecutively from the case load of the chest clinics of the New York City Health Department between May 1, 1955, and April 30, 1956, in the order in which they came to the writers' attention and without any attempt at exhaustive coverage of the entire suitable case material available in the clinics at the time. A special system was devised wherein all clinic patients classified as having disease "arrested with cavitation" were registered with the writers for review.

For a case to be included in the study, it had to fulfill all of the following criteria:

(*1*) A past diagnosis of active, bacteriologically confirmed pulmonary tuberculosis

(*2*) A period of at least six months of antituberculous drug therapy

[1] From the Bureau of Tuberculosis, New York City Department of Health, New York, New York.

(*3*) Achievement, under or following drug therapy, of a clinical status characterized by:

a. The presence of at least one air space on the roentgenogram (conventional or sectional), clearly enough outlined to be measurable on some films and at least 1 cm. in its greatest diameter[2]

b. At least six months' stability on roentgenogram of all noncavitary components of the disease, and

c. At least six consecutive cultures negative for tubercle bacilli from sputum, laryngeal swab, or gastric contents obtained during the period of roentgenographic stability

(*4*) A period of observation[3] of at least six months subsequent to achievement of the status defined in criterion (*3*), i.e., patients with disease that showed "arrest with cavitation" were still excluded from the study if a minimum of six months' subsequent observation was not available.

The roentgenograms and clinic records of patients were reviewed periodically. The last such review of the case material was carried out in December, 1957.

Reactivations were diagnosed on the basis of positive bacteriologic findings for tubercle bacilli or extensions in the *noncavitary* components of the disease on roentgenogram. Enlargement of a cavity per se or development of a fluid level therein were not considered evidences of relapse.

Composition of group: The breakdown of the patient group according to age, race, and sex is given in the first four columns of table 1.

Seventy-three per cent of the patients in the study group were male, two-thirds of the males being forty-five years of age or older, and none less than twenty-five. Twenty-seven per cent were females, only one-fourth of them being forty-five years of age or older. Thirty-eight per cent of the patients were white, the age groups of more than forty-five years predominating, while the majority of the 49 per cent of Negro patients and 13 per cent of Puerto Rican-born patients were less than forty-five years of age.

Past history: Available data of past histories varied widely with respect to degree of detail and reliability. Even on such crucial points as type, dosage, and duration of drug therapy, information was often incomplete or uncertain. There would be nothing to gain, therefore, from any attempts at refining the historic material. Suffice it to say that: Almost all patients had had far advanced disease; of the 94 patients, 77 had been hospitalized for three months or more since January, 1950; and despite wide variation in drug combinations, schedules, and dosages, all patients in the study received isoniazid at some time or other in the course of their disease.

Duration of observation[3]*:* The duration of arrest in 10 patients whose disease reactivated during the period of study ended with the date of reactivation. This will be discussed under the heading "Results."

Among the 84 patients whose disease was still classified as arrested at the close of the study, the length of observation was as follows (table 2):

6 to 11 months in 4 cases
12 to 23 months in 17 cases
24 to 35 months in 29 cases
36 months or more in 34 cases

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[2] In the case of air spaces with the appearance of emphysematous bullae, evidence was also required that their presence did not antedate drug therapy. In the absence of such evidence, the case was excluded from the study as one of presumably ordinary emphysematous bullae. Multiple highlights compatible with a diagnosis of post-tuberculous bronchiectasis were also excluded from the study.

[3] The term "start of observation" will be used hereafter to designate the time when a case first reached the status defined in criterion (*3*) under "Materials and Methods."

TABLE 1

NUMBER AND PER CENT OF REACTIVATIONS BY AGE, SEX, AND ETHNIC GROUP AMONG 94 PATIENTS WITH PERSISTENT CAVITATION AND NONINFECTIOUS SPUTUM

Age and Sex	Total by Ethnic Group				Number Relapsed by Ethnic Group Total				Per Cent Relapsed by Ethnic Group			
	Total	White	Negro	Puerto Rican	Total	White	Negro	Puerto Rican	Total	White	Negro	Puerto Rican
Total	94	36	46	12	10	6	3	1	11	16	6	8
Male	60	33	30	6	9	6	3	0				
Less than 25	—	—	—	—	—	—	—	—				
25–44	22	8	11	3	2	1	1	0				
45–64	38	19	17	2	7	5	2	0				
65 or older	9	6	2	1	0	0	0	0				
Female	25	3	16	6	1	0	0	1				
Less than 25	1	—	—	1	0	—	—	0				
25–44	18	1	13	4	1	0	0	1				
45–64	4	1	2	1	0	0	0	0				
65 or older	2	1	1	—	0	0	0	—				

TABLE 2

OUTCOME, BY DURATION OF ARREST

Months of Arrest	Total Patients	Disease Remained Arrested	Disease Relapsed
0–5	4	0	4
6–11	6	4	2
12–17	9	6	3
18–23	11	11	—
24–29	16	15	1
30–35	14	14	—
36 and older	34	34	—
Total	94	84	10

RESULTS

Tuberculosis reactivations: Of the total group of 94 patients, 10 suffered reactivation of their disease during the period of observation, one death resulting from active tuberculosis (table 1). The intervals from "start of observation" to reactivation of the tuberculosis were as follows: In 6 patients the disease reactivated during the first year of observation; in 3 patients reactivation occurred during the second year of observation; and in one patient the disease reactivated during the third year of observation (table 2).

In 3 of these 10 cases, the diagnosis of reactivation was based on roentgenographic changes alone; in 3, on bacteriologic findings alone; in 3, on both roentgenographic changes and bacteriologic findings; and in one case the reactivation was reported by an outside agency, the basis for the diagnosis not being stated. All but one reactivation occurred within eighteen months of the "start of ob-

TABLE 3

CAVITY CHANGE SINCE "START OF OBSERVATION," BY OUTCOME

Cavity Change	Total Patients	Disease Remained Arrested	Disease Relapsed
Disappeared	6	6	—
Contracted	15	14	1
No change	62	56	6
Enlarged	5	3	2
Fluid	7	6	1
Unstable	5	4	1
Total	100*	89*	11*

* Some patients had more than one kind of cavity change.

servation," and all but one of the patients were reported as still receiving drug treatment at the time. As with any patients on self-administered drug treatment, the patients in the present study may not have taken their drugs regularly, or may have stopped medication without so informing their physicians. In one additional patient, relapse occurred two months after discontinuance of drug treatment.

Cases of disease remaining inactive: Among the 84 patients whose disease remained noninfectious and stable in its noncavitary components, cavities were no longer seen at the end of observation in 6 cases. The cavities contracted in 14 cases, enlarged in 3 cases, showed irregular instability in 4 cases, and showed a fluid level in 6 cases (table 3). There were 6 deaths in this group from the following causes: 2 from coronary arterial disease, and one each from perforated peptic ulcer, carcinoma of the stomach, suppurative pneumonia, and suicide.

Among the 84 patients, 56 were still receiving drug treatment at the close of the study. Seven of the remaining 28 patients were observed for at least three years following discontinuance of drug treatment; an additional 8, for at least two years; and an additional 7, for at least one year. Thus 22 patients remained noninfectious with stable pulmonary lesions for periods of one year or more after completion of the chemotherapy.

Reactivation of disease was found to be uncommon in patients whose cavities had thin walls or large diameters (5 cm. or more) (*see* tables 5 and 8).

DISCUSSION

If useful information is to come from a study such as the present one, attempts must be made to discover whether any factors known at the "start of observation" can be successfully related to the subsequent behavior of the disease in terms of stability or tendency to relapse. Such factors can be looked for among the patient's biologic characteristics, his past tuberculous history (including length and type of treatment), and the roentgenographic appearance of his pulmonary lesions.

Unfortunately, available past histories were too fragmentary, and information concerning type of drug therapy was too uncertain, to permit tabulation of this

TABLE 4

OUTCOME BY NUMBER AND DISTRIBUTION OF CAVITIES

Number of Cavities	Total Patients	Disease Relapsed
Single	34	2
Multiple-unilateral	28	3
Multiple-bilateral	32	5
Total	94	10

sort of information in the present study. No information was obtained from attempts to relate frequency of a relapse to symptoms or to length of drug treatment administered either prior, or subsequent, to the "start of observation." Moreover, the number of patients in the present series was too small to permit any but the most tentative conclusions. Despite these shortcomings, the data presented do suggest certain trends.

No relationship was found in the study group between frequency of relapse and extent of noncavitary lung involvement. One-half of all reactivations occurred among the 32 patients with multiple bilateral cavitation, as may be seen in table 4. On the other hand, rather striking differences in frequency of relapse were noted when the patient-material was grouped according to any of the following characteristics: sex, age, race, thickness of cavity wall,[4] and cavity size.

For easy comparison, it may be permissible, despite the small numbers involved, to quote here the following rates of relapse:

Relapse occurred in 13 per cent of the 69 males and in 4 per cent of the 25 females; it occurred in 13 per cent of the 53 persons more than forty-five years of age and in only 7 per cent of the 41 patients less than forty-five years old. Among the 36 white patients, relapse of the tuberculosis was noted in 17 per cent, whereas it occurred in only 7 per cent of the 46 Negro patients. (In a small subgroup of 12 Puerto Rican-born patients, relapse occurred in 8 per cent.) Relapse occurred in 19 per cent of the 42 persons with cavities designated as "other than thin-walled" and in only 4 per cent of the 52 patients with "thin-walled" cavities. With respect to cavity size, relapse appeared in 14 per cent of the 64 persons with cavities 1 to 5 cm. in diameter and in 3 per cent of the 30 persons with cavities 5 cm. or more in diameter.

Eight of the 10 reactivations occurred in patients whose cavities were classified as "other than thin-walled" (table 5). The biologic groups that had the high rates of reactivation (the male, the elderly, the white) all contained a correspondingly high proportion of "other than thin-walled" cavity cases when compared to the complementary groups (the female, the young, the Negro). Once this fact was taken into account and comparison was restricted to the "other

[4] Cavities were called "thin-walled" only if the entire visible portions of all distinguishable cavity walls appeared of hairline or nearly hairline thickness. Whenever there was doubt on this point or if the thickness of cavity walls varied from one location to another, or from one roentgenographic view to another, such cavities were classified, together with the obviously thick-walled ones, as "other than thin-walled."

TABLE 5

OUTCOME, BY THICKNESS OF CAVITY WALL

Thin Cavity Wall	Total Patients	Disease Remained Arrested	Disease Relapsed
Yes	52 (100.0)	50 (96.1)	2 (3.9)
No	42 (100.0)	34 (81.0)	8 (19.0)
Total	94 (100.0)	84 (89.4)	10 (10.6)

TABLE 6

RELAPSES AMONG PATIENTS WITH "OTHER THAN THIN-WALLED" CAVITIES BY AGE, SEX, AND ETHNIC GROUP

	Total			Relapses		
	Total	White	Negro and Puerto Rican	Total	White	Negro and Puerto Rican
Total	42	22	20	8	5	3
Males	36	21	15	7	5	2
Less than 45	10	4	6	2	1	1
45 or older	26	17	9	5	4	1
Females	6	1	5	1	—	1
Less than 45	4	0	4	1	—	1
45 or older	2	1	1	—	—	—

than thin-walled" cases (table 6), no essential differences were noted any longer between the relapse rates in males and females, in the elderly and in the young, or in the white as compared with the non-white. It seems reasonable, therefore, to explain differences of relapse rates in these biologic categories by the corresponding differences in the prevalence of "other than thin-walled" cavities. Thin-walled cavity cases were much more frequent in the female, the young, the Negro, and the Puerto Rican group than in the male, the elderly, and the white group (table 7).

The relatively high relapse rate among the patients with small, as compared with those with large, cavities also appears related to thickness of cavity wall (table 8). Of the 30 patients with large cavities, 77 per cent had thin-walled cavities, and their disease did not relapse. Only 23 per cent of the large cavities were in the "other than thin-walled" group, and there was one relapse among them.

On reviewing serial roentgenograms for this study, it was a common experience to see the walls of cavities first "thin out" under treatment until a bulla-like appearance was reached. These bullous cavities subsequently either remained permanently unchanged, or they showed more or less rapid and unpredictable changes in size in either direction. The changes would lead either to shrinkage and disappearance of the cavity or to the formation of large tension bullae. Either way, the behavior of the cavity strongly suggested the presence of a bronchiolar obstruction. Such cavity behavior was usually observed to

TABLE 7

COMPARISON, AS TO FREQUENCIES OF THIN-WALLED AND "OTHER THAN THIN-WALLED" CAVITY CASES, BETWEEN THE SEXES, THE AGE GROUPS, AND THE ETHNIC GROUPS

	Sex		Age		Ethnic Group	
	Male	Female	Less than 45	45 or older	White	Negro or Puerto Rican
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Thin-walled	48	76	66	47	39	66
"Other than thin-walled"	52	24	34	53	61	34
Total	100	100	100	100	100	100

TABLE 8

NUMBER AND PER CENT OF RELAPSES BY CAVITY SIZE AND THICKNESS OF CAVITY WALL

Diameter and Thickness of Wall	Total	Number of Relapses	Per Cent of Relapses
1–5 cm.	64	9	14
Thin-walled	29	2	7
Other than thin-walled	35	7	20
5 cm. or more	30	1	3
Thin-walled	23	0	0
Other than thin-walled	7	1	14
Total	94	10	11

occur in lung portions that had shown a more acute pneumonic type of tuberculous involvement. This circumstance might account, at least in part, for the previously mentioned sex, age, and ethnic difference in frequency of thin-walled cavities. In some instances, bullous formations that developed and changed size under drug treatment could not be traced back to any demonstrably pre-existing tuberculous cavities. Shrinkage or ballooning of cavities occurred most typically in the earlier phases of drug treatment and had often ceased by the time of "start of observation." Since available records of this earlier phase of the disease were rather incomplete, no attempt was made to report them here. Such changes are shown in table 3 only in as much as they occurred *after* the "start of observation." The same holds true for the presence of fluid levels which, when noted only prior to the "start of observation," would not appear in table 3.

Of 7 patients who showed fluid levels after the "start of observation," only one subsequently showed reactivation of disease. But the patient who died from suppurative pneumonia (as proved by autopsy) had had very extensive bulla-like cavity formations throughout both lungs, with alternating (and often simultaneous) ballooning of some cavities, shrinkage or disappearance of others, and intermittent presence of fluid levels. Obviously, these cavities were the sites of recurrent nonspecific infections and may well have been the source of the terminal pulmonary infection.

In one of the patients, extensive formation of bulla-like cavities was associated with a severe degree of pulmonary insufficiency. Spontaneous pneumothorax not associated with reactivation occurred in another patient.

To recapitulate: The incidence of reactivation of disease found in this group of ambulatory tuberculous patients with cavities and noninfectious sputum was 11 per cent. Nine of 10 relapses occurred within eighteen months of the "start of observation." The observation periods of the patients whose disease did not reactivate averaged about two and a half years.

The thickness of the residual cavity walls on the roentgenogram may turn out to be a prognostically useful criterion in that it divided this study group into subgroups with relapse rates as widely apart as 4 per cent for thin-walled, and 19 per cent for "other than thin-walled," cavity cases. Consequently, the presence of thick-walled residual cavities, barring other reasons to the contrary, would constitute a fairly strong indication for resectional surgery. With the thin-walled cavity cases, a wait-and-see attitude seems to be much more justifiable, although factors such as nontuberculous infection, spontaneous pneumothorax, or giant tension cavity may add to the indications for surgical intervention.

Summary

Ninety-four ambulatory patients with tuberculous cavities, stable roentgenographic findings, and sputum free of tubercle bacilli following drug treatment were consecutively selected by definite criteria from the clinic rolls of the New York City Department of Health between May 1, 1955, and April 30, 1956. They were followed clinically for periods averaging approximately two and one-half years from the time when roentgenographic stability and noninfectiousness of sputum had both been present for at least six months.

Among this series, the disease in 84 patients remained arrested, while 10 experienced reactivations of their disease, one death resulting from active tuberculosis. Nine of the reactivations occurred within eighteen months from the time when the criteria of roentgenographic stability and noninfectious sputum were first fulfilled.

Two of the 10 observed relapses occurred in patients whose cavities were classified as "thin-walled," 8 in those with "other than thin-walled" cavities. This gave a relapse rate of 4 per cent among the thin-walled cavity cases, and of 19 per cent among the others.

Differences in relapse rates between sexes, age groups, and racial and geographic groups were accounted for by corresponding differences in the frequencies of "other than thin-walled" cavities.

It is suggested that thick-walled cavities have a fairly serious prognosis with respect to reactivation of tuberculous disease, and may be considered indications for resection. It is further suggested that the presence of a thin-walled cavity per se may not have as much weight in a decision relating to surgery except when the intent is to eliminate, not only a possible focus of reactivation,

but also a potential source of nonspecific infection, or a possible cause of spontaneous pneumothorax or the physiologic hazards of a tension cavity.

Sumario

Observaciones en los Enfermos Ambulantes con Cavernas Tuberculosas "Negativas Abiertas"

Noventa y cuatro enfermos ambulantes que tenían cavernas tuberculosas, radiografías estabilizadas y cultivos de esputo negativos para bacilos tuberculosos a continuación de la farmacoterapia fueron escogidos consecutivamente conforme a pautas precisas de los registros de las clínicas del Departamento de Sanidad de la Ciudad de Nueva York entre el 1º de mayo de 1955 y el 30 de abril de 1956. Se les mantuvo en observación clínica durante períodos de no menos de ocho meses que promediaron dos años y medio, contando desde la fecha en que habían existido estabilidad radiográfica y falta de infecciosidad del esputo a lo menos seis meses.

En esta serie, la enfermedad permaneció estacionada en 84 enfermos, en tanto que 10 enfermos experimentaron reactivaciones de su dolencia, con una muerte debida a tuberculosis activa. Nueve de las reactivaciones tuvieron lugar en término de dieciocho meses de la fecha en que por primera vez se cumplieron las pautas de estabilidad radiográfica y negatividad de los cultivos con respecto a bacilos tuberculosos.

Dos de las 10 recidivas observadas correspondieron a enfermos cuyas cavernas se habían clasificado como "de paredes delgadas" y 8 en sujetos que tenían cavernas que no eran "de paredes delgadas". Esto arrojó un índice de 4 por ciento de recidivas entre los casos de cavernas de paredes delgadas y de 19 por ciento en los demás.

Las diferencias en los índices de recidividación entre los sexos, los grupos de edades y los grupos étnicos y geográficos encontraron su explicación en correspondientes diferencias en la frecuencia de cavernas que no eran "de paredes delgadas."

Apúntase que las cavernas de paredes gruesas comportan un pronóstico bastante grave con respecto a reactivación de la enfermedad tuberculosa y pueden considerarse como indicaciones para la resección; y que la existencia de una caverna de paredes delgadas tal vez no sea de mucho peso en la decisión relativa a cirugía, excepto cuando el objeto es eliminar, no sólo un posible foco de reactivación, sino también un foco potencial de infección anespecífica o una posible causa de neumotórax espontáneo.

Resume

Observations chez des malades ambulatoires porteurs de lésions cavitaires tuberculeuses "ouvertes négatives"

Quatre-vingt-quatorze malades ambulatoires, porteurs de cavernes tuberculeuses avec radiogrammes stables et cultures de crachats négatives pour le BK après chimiothérapie, ont été choisis en série au moyen de critères définis parmi l'ensemble des malades des cliniques du Département de la Santé Publique de l'Etat de New York entre le 1er mai 1955 et le 30 avril 1956. Ils ont été suivis cliniquement pendant des périodes qui n'étaient pas inférieures à huit mois et atteignaient en moyenne environ deux ans et demi. Ces périodes étaient comptées à partir du moment où la stabilisation radiologique et la "négativation" des crachats étaient acquises pendant un minimum de six mois.

Dans cette série, la maladie est demeurée stabilisée chez 84 malades, 10 malades ont présenté une réactivation de l'infection, un malade a succombé à une tuberculose évolutive. Neuf des réactivations se sont produites dans les dix-huit mois suivant le moment où les critères relatifs à la stabilisation radiologique et à la négativation des cultures de l'expectoration ont été primitivement acquis.

Deux des dix rechutes observées concernaient des cas où existaient des cavernes de la catégorie "paroi mince", huit cas appartenaient à d'autres catégories que celle des cavernes à paroi mince. Ceci correspondait à un taux de rechute de 4% parmi les cas à "paroi mince" et à 19% parmi les autres.

Les différences dans les taux de rechute entre les sexes, groupes d'âge, groupes raciaux ou géographiques sont rapportées à des différences correspondantes dans la fréquence des cavernes appartenant à une autre catégorie que celle à "paroi mince".

Les auteurs suggèrent que les cavernes à paroi épaisse sont d'un pronostic assez grave à l'égard de la réactivation de l'infection tuberculeuse et peuvent être considérées comme indication de la résection. D'autre part, la présence d'une caverne à paroi mince ne peut, à elle seule, peser d'un même poids dans la décision de recourir à la chirurgie, sauf lorsqu'il s'agit d'éliminer non seulement un foyer possible de réactivation, mais aussi une source potentielle d'infection non spécifique ou une cause éventuelle de pneumothorax spontané.

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BACTERIOLOGIC AND CLINICAL SIGNIFICANCE OF THE CATALASE ACTIVITY OF MYCOBACTERIUM TUBERCULOSIS[1]

OTTO SCHWEIGER AND EDIT VANDRA

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INTRODUCTION

The use of antimicrobials, especially isoniazid, has led to morphologic and biologic changes in Mycobacteria. One such change is the loss of catalase activity of tubercle bacilli, a phenomenon first demonstrated by Middlebrook (1*a*). In addition, the isoniazid-resistant strains were found to have lowered virulence for the guinea pig (1*b*, 2–5*b*, and others). Subsequent to these observations, studies were initiated to establish the effect of prolonged isoniazid therapy on both the virulence of tubercle bacilli excreted by such patients and the clinical course of the patients' disease. Oestreicher and associates (6) reported favorable results in cases of advanced tuberculous patients treated with isoniazid for a year. Such optimistic views, however, were not shared by Rist (7*a*, *b*), and Trendelenburg (8). In animal experiments and clinical studies, these writers have shown that isoniazid-resistant strains with decreased virulence for the guinea pig might still be harmful for humans, as demonstrated by the fact that caseous lesions were seen to develop in some of their cases.

In the present study, a trial has been made to determine the clinico-pathologic significance of isoniazid-resistant strains with lowered virulence for the guinea pig.

MATERIAL AND METHODS

The group reported in this study consisted of 20 patients with advanced, cavitary tuberculous disease. At the beginning of the observation period, the sputum of all of the patients revealed tubercle bacilli on microscopy, and all had previously had long courses of antimicrobial therapy with large amounts of isoniazid. At monthly intervals during the three months of observation, sputum specimens from these patients were cultured for tubercle bacilli, and the roentgenographic and clinical status was evaluated. The patients were observed clinically for more than a year.

The standard method, for bacteriologic culturing as modified by Szabó (9), was used. According to this method, homogenization was done by the use of trinatrium phosphate with aluminium phosphate added in order to obtain sufficient sedimentation. Then, aliquots of the resulting sediment were inoculated into two tubes of Löwenstein-Jensen medium, two tubes of liquid medium (Sula medium (10)), and two tubes of Löwenstein-Jensen medium which also contained 10γ of isoniazid per ml. The purpose in using the medium combined with isoniazid was to obtain during the primary culturing quantitative information about the relative proportion of isoniazid-susceptible and isoniazid-resistant colonies. The cultures were read after incubation at 37°C. for four, six, and eight weeks. The strains grown in the isoniazid-containing medium were subcultured twice in a medium of a similar composition in order to inhibit the growth of any isoniazid-susceptible bacilli possibly present in the primary culture. Thus, two types of strains were obtained from each of the patients: the first type had a mixed bacterial population, containing both isoniazid-sus-

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ceptible and -resistant bacilli, and the second type contained only resistant bacilli. Both types of strains were then subjected to the following tests in parallel: an isoniazid-resistance test; a cytochemical test; a catalase-activity test; and guinea pig inoculation.

In carrying out the tests the following methods were used:

Isoniazid-resistance test: Colonies from the liquid or solid medium were placed in tubes containing 2 ml. of liquid medium and were homogenized. Care was taken to obtain an approximately identical viscosity of the suspension in each of the cases. One to two drops from this suspension were then inoculated into a medium with graded concentrations of isoniazid. The grades of isoniazid were as follows: 1.0, 2.5, 5.0, 10.0, 20.0, 40.0, 80.0 γ per ml. The test was read after incubation at 37°C. for three weeks. Two tubes of medium containing no drug were used as controls in each of the tests.

Cytochemical test: The cultures were subjected to the cytochemical tests of Dubos, Desbordes, and Wilson. The technique used for these tests was the method described by Paraf and associates (11).

Catalase-activity tests: Cultures from the liquid medium were used for the catalase activity tests. After elimination of the medium by filtration, the bacterial colonies were washed thrice with physiologic saline. A loopful from the bacilli was suspended in 1 ml. buffer of pH 7.0 and pH 1.0, respectively (Sörensen phosphate and citrate buffer were used). Then 1 ml. of 0.70 per cent hydrogen peroxide was added to this suspension. The tubes were shaken, left at 20°C. for thirty minutes, and then examined for the production of air bubbles, the indication of catalase activity.

Animal inoculation: The cultures used for animal inoculation were four weeks old and were suspended in saline to give a suspension containing 0.01 mg. of culture per ml. One milliliter of this suspension was injected subcutaneously in the left inguinal region of a pair of guinea pigs. Control guinea pigs injected with the virulent H37Rv strain were also used. The animals were sacrificed at the end of the eighth week after inoculation, sectioned, and the lesions were classified according to the degree of severity as follows:

Absence of lesions even at the site of the inoculation

Enlargement and partial caseous necrosis of the regional lymph nodes corresponding to the site of inoculation

Enlargement of the regional lymph nodes with tuberculous lesions in the spleen

Tissue changes in the liver as well as in the lymph nodes and the spleen

Evidence of generalized tuberculosis in the spleen, the liver, and the lungs

Results

Bacteriologic Tests

Generally, the growth of the bacilli was found to be slower in the medium containing isoniazid than in the isoniazid-free medium.

As a rule, abundant growth with "numerous" colonies was seen to develop after eight weeks of incubation in the medium containing isoniazid, and after four to six weeks of incubation in the isoniazid-free medium. Three strains which proved to be susceptible to isoniazid developed numerous colonies in the isoniazid-free medium. In the medium containing isoniazid, 2 of these 3 strains showed a decreased number of colonies, and one continued to produce a large number of colonies. In 3 additional cases, an increase in the number of colonies was found in the isoniazid-containing medium as compared with the isoniazid-free medium. All of these 3 strains exhibited a high level of resistance to 80 and 40 γ of isoniazid per ml.

Great differences were found in the results of the cytochemical tests performed. On the basis of the examinations, no correlation could be demonstrated between

TABLE 1

RELATIONSHIP OF DEGREE OF ISONIAZID RESISTANCE TO CATALASE ACTIVITY AND TO VIRULENCE FOR GUINEA PIGS

Detailed Data on Individual Cases

	Culture Medium Without Isoniazid				Culture Medium With Isoniazid			
	Isoniazid Resistance*	Catalase Activity*†		Virulence‡	Isoniazid Resistance*	Catalase Activity†		Virulence‡
Case 1, H. K.	20	Positive	Positive	4.0	80	Negative	Negative	3.0
	20	Positive	Positive	4.0	20	Negative	Negative	4.5
	5	Positive	Negative	2.5	20	Negative	Negative	2.5
Case 2, M.J.	20	Negative	Negative	3.0	80	Negative	Negative	1.0
	40	Negative	Negative	2.5	80	Negative	Negative	1.5
	20	Negative	Negative	—	40	Negative	Negative	—
Case 3, P.J.	20	Positive	Negative	2.5	20	Negative	Negative	1.5
	40	Positive	—	3.5	10	Negative	Negative	2.0
	20	Positive	Negative	2.0	20	Negative	Negative	1.0
Case 4, H.K.	Susceptible	Positive	Negative	5.0	20	Negative	Negative	2.0
	—	—	—	—	—	—	—	—
	20	Positive	Negative	—	10	Negative	Negative	1.0
Case 5, A.R.	5	Negative	Negative	1.0	80	Negative	Negative	1.5
	10	Negative	Negative	1.0	80	Negative	Negative	1.0
	20	Negative	Negative	—	40	Negative	Negative	—
Case 6, L.J.	20	Negative	Negative	3.5	20	Negative	Negative	3.0
	10	Positive	Negative	—	40	Negative	—	1.0
	20	Negative	Negative	1.0	20	Negative	Negative	—
Case 7, B.F.	10	Positive	Negative	2.0	80	Negative	Negative	3.0
	40	Positive	Negative	3.0	40	Negative	Negative	1.0
	20	Negative	Negative	1.0	10	Negative	Negative	4.0
Case 8, H.Á.	5	—	—	—	—	—	—	—
	5	Positive	—	5.0	10	Positive	Positive	4.0
	10	Positive	Negative	5.0	—	—	—	1.0
Case 9, K.M.	Susceptible	—	—	4.5	40	Positive	Negative	5.0
	10	Positive	Positive	4.5	10	Positive	Negative	1.0
	5	Positive	Positive	5.0	—	—	—	—
Case 10, P.I.	—	—	—	—	—	—	—	—
	20	Positive	Positive	5.0	20	Positive	Positive	4.0
	—	—	—	—	—	—	—	—
Case 11, T.I.	10	Positive	—	2.0	80	Negative	Negative	1.0
	10	Positive	Negative	3.0	40	Negative	Negative	—
	10	Negative	Negative	1.0	10	Negative	Negative	—
Case 12, U.J.	—	—	—	—	—	—	—	—
	5	Negative	Negative	1.0	10	Negative	Negative	2.5
	—	—	—	—	—	—	—	—
Case 13, H.I.	10	—	—	—	—	—	—	—
	Susceptible	Positive	Negative	3.5	20	Positive	Positive	1.0
	5	—	—	—	—	—	—	—

* Isoniazid resistance in γ per ml.

† Catalase activity at pH 7.0 and 1.0, respectively.

‡ Virulence for guinea pigs. The numerical values indicate the degree of severity of the lesions. (*See* text, "Animal inoculation.")

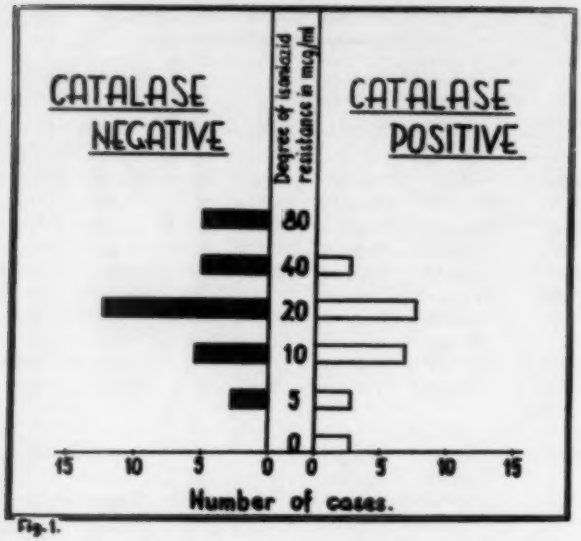

FIG. 1. The relationship of isoniazid resistance to catalase activity on the basis of the data shown in table 1.

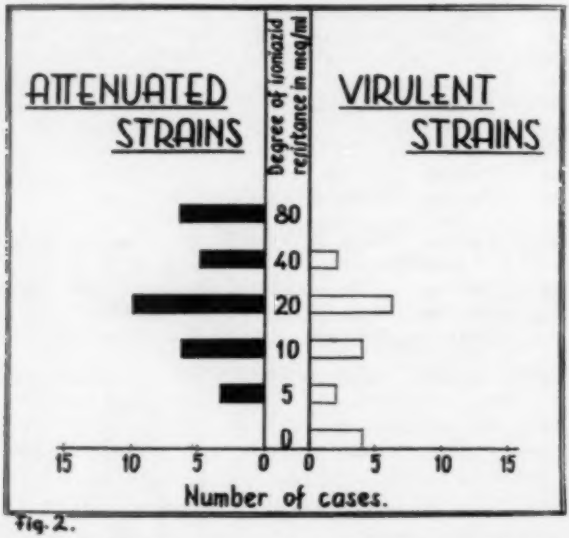

FIG. 2. The relationship of isoniazid resistance to virulence for the guinea pig on the basis of the data shown in table 1.

the results of cytochemical tests and animal experiments, on the one hand, and between cytochemical reactions, the level of isoniazid resistance, and catalase-activity tests, on the other hand.

The relationship of the results of animal experiments to isoniazid resistance and catalase activity of the strains studied may be seen in table 1.

That the isoniazid-resistance values are highly variable is shown in that table. The resistance values were found to vary not only from one patient to another, but significant differences were also seen in the results of the various tests performed on the same patient.

No correlation was found between the results of resistance tests performed on various occasions.

In comparing the resistance values of the bacilli cultured in the isoniazid-free medium with those from the isoniazid-containing medium, 3 strains from the isoniazid-free medium were found to be drug susceptible. On the other hand, the strains from the isoniazid-containing medium showed higher levels of resistance than the strains from the isoniazid-free medium.

The relationship of isoniazid resistance to catalase activity based on the data in table 1 is shown in figure 1.

In figure 1 it may be seen that all of the 3 drug-susceptible strains showed catalase activity. On the other hand, the strains with resistance levels over 80 γ of isoniazid per ml. proved to be catalase negative, while the strains with resistance levels of 5 to 10 γ of isoniazid per ml. were equally catalase positive and catalase negative. Catalase-negative strains predominated among the strains with 20-to-40 γ per ml. resistance levels.

The relationship of isoniazid resistance to guinea pig virulence is shown in figure 2.

From figure 2 it is evident that all of the drug-susceptible strains were virulent for the guinea pig. In contrast, the strains resistant to more than 80 γ per ml. of isoniazid were found to be of lowered virulence, and the attenuated strains predominated among the strains with resistance levels of 5, 10, 20, and 40 γ per ml.

The summary of the results on catalase activity and virulence for the guinea pig, as presented in table 1, is shown in table 2. It may be seen that the majority of catalase-positive strains proved to be virulent for the guinea pig. In contrast, only 2 of the 26 catalase-negative strains were found virulent for the guinea pig; the remaining 24 strains showed lowered virulence.

In addition, the question was studied whether there was a difference in the degree of guinea pig virulence of strains showing catalase activity at both pH 1.0 and pH 7.0, on the one hand, and those showing catalase activity at pH 7.0, but catalase negativity at pH 1.0. The results are shown in table 3. It may be seen that only 2 of the 14 strains which were catalase positive at both pH 7.0 and pH 1.0 showed decreased virulence for the guinea pig. In contrast, 5 of the 9 strains which were catalase negative at pH 1.0 showed decreased virulence for the guinea pig.

TABLE 2

SUMMARY OF THE RESULTS ON CATALASE ACTIVITY AND VIRULENCE FOR GUINEA PIGS AS PRESENTED IN TABLE 1

Catalase Activity	Virulent Strains	Attenuated Strains
Positive	17	8
Negative	2	24

TABLE 3

RELATIONSHIP OF GUINEA PIG VIRULENCE TO CATALASE ACTIVITY AT pH7 AND pH1

Catalase Activity		Virulent Strains	Attenuated Strains
Positive	pH7 }	12	2
	pH1 }		
Positive	pH7 }	4	5
Negative	pH1 }		

Clinical Examinations

As stated previously, the patients selected for the study had far advanced pulmonary tuberculosis and had previously received long courses of isoniazid (5 to 10 mg. per kg. daily). They were maintained on isoniazid therapy during the period of the study. Isoniazid-resistance tests, catalase-activity tests, and virulence tests were performed at monthly intervals for three months. The patients were followed clinically for more than a year.

Seven of the original 20 patients were excluded from the study because of failure to obtain positive cultures from them in all of the tests. A study with similar methods but on a much greater patient group is in progress.

DISCUSSION

On the basis of the evidence gained from the present material, it was possible to confirm the observations made by several writers on the relationship between the isoniazid resistance, catalase activity, and guinea pig virulence of tubercle bacilli (1*a*–*c*, 5*b*, 12–21).

It was found in the present study that the number of the catalase-negative strains increases in direct proportion to the increase in the level of isoniazid resistance. Among the strains which were resistant to concentrations as high as 80 γ of isoniazid per ml., only catalase-negative strains were found (22–24 and others.) The fact that the majority of strains cultured in the isoniazid-containing medium proved to be catalase negative provides additional proof for the existence of a correlation between isoniazid resistance and catalase activity. It was found that the increase in the isoniazid-resistance level ran parallel to the decrease in the catalase activity of the tubercle bacilli.

As mentioned above, significant differences were found in the isoniazid-resistance level between strains grown in the isoniazid-free and the isoniazid-containing medium, respectively. In one of the cases observed, such a difference was as high

as a variation from isoniazid susceptibility to resistance to 40 γ of isoniazid per ml. In other cases, resistance was found to vary from 5 to 80 γ per ml. In addition, similarly high differences were seen between the results of tests performed at various periods of time. This phenomenon was equally observed in strains from isoniazid-free and isoniazid-containing media.

In strains with a mixed population of isoniazid-resistant and isoniazid-susceptible bacilli, identical values were never obtained in the tests performed at different periods of time. The case was essentially the same with the resistant population except in the strain from one patient (*A.R.*) in whom identical values were obtained in all of the tests.

Similar differences were also seen in the results of the catalase and virulence tests. Not infrequently, the same patient excreted virulent and catalase-positive strains in the first month of observation and yielded only catalase-negative and avirulent strains in the next month.

In general, the following variations were observed in the catalase activity of sputum strains from a patient: only catalase-positive strains; only catalase-negative strains; a preponderance of catalase-positive strains; a preponderance of catalase-negative strains; and, finally, an identical proportion of both catalase-positive and catalase-negative strains.

It is believed that the importance of the above observations, lies in the fact that the bacterial populations isolated from tuberculous patients treated with isoniazid cannot be taken as constant or homogenous in composition. This fact was already pointed out by Rist (7*b*), who was able to culture from sputum strains infected into guinea pigs a bacterial population containing isoniazid-susceptible, isoniazid-resistant, catalase-positive, and catalase-negative bacilli. Perhaps it is this phenomenon which may account for the divergences in the results obtained by the various writers, and the differences between laboratory resistance data and clinical observation of the patient. It would appear that the bacterial population in the sputum is highly diversified in composition, and the isoniazid-resistance data as given by the laboratories are inadequate to show the true composition of the population. In addition, the results of the present study point to the fact that a great number of consecutive catalase-activity tests are needed before the true character and composition of the bacterial population of a patient can be estimated. A few tests are in no way sufficient to provide conclusions as to the composition of a patient's strain.

In order to study the possible significance of the bacteriologic findings obtained, the patients were divided into several groups according to the clinical course of their disease, and the bacteriologic results were considered in relation to each of these groups. Clinically, it was possible to distinguish the group of patients with continuously good clinical condition, the group with clinical progression of their disease, and the group of patients who died. The results for each of these groups are shown in tables 4–6, respectively.

The results of patients in good clinical condition are demonstrated in table 4. This group included patients in whom no evidence of tuberculous progression was detected roentgenographically during the period of observation, despite persisting cavities and the continous discharge of tubercle bacilli in the sputum.

TABLE 4

PATIENTS WITH GOOD CLINICAL CONDITION CONTINUOUSLY

	Isoniazid Resistance		Catalase Activity		Virulence	
	Medium Without Isoniazid	Medium With Isoniazid	Medium Without Isoniazid	Medium With Isoniazid	Medium Without Isoniazid	Medium With Isoniazid
Case 2, M.J.	20	80	Negative	Negative	3.0	1.0
	40	80	Negative	Negative	2.5	1.5
	20	40	Negative	Negative	—	—
Case 5, A.R.	5	80	Negative	Negative	1.0	1.5
	10	80	Negative	Negative	1.0	1.0
	20	40	Negative	Negative	—	—
Case 13, H.I.	10	—	—	—	—	—
	Susceptible	20	Positive	Positive	3.5	1.0
	5	—	—	—	—	—
Case 6, L.J.	20	20	Negative	Negative	3.5	3.0
	10	40	Positive	Negative	—	1.0
	20	20	Negative	Negative	1.0	—
Case 12, U.J.	—	—	—	—	—	—
	5	10	Negative	Negative	1.0	2.5
	—	—	—	—	—	—
Case 7, B.F.	10	80	Positive	Negative	2.0	3.0
	40	40	Positive	Negative	3.0	1.0
	20	10	Negative	Negative	1.0	4.0

TABLE 5

PATIENTS WITH CLINICAL PROGRESSION

	Isoniazid Resistance		Catalase Activity		Virulence	
	Medium Without Isoniazid	Medium With Isoniazid	Medium Without Isoniazid	Medium With Isoniazid	Medium Without Isoniazid	Medium With Isoniazid
Case 1, H.K.	20	80	Positive	Negative	4.0	3.0
	20	20	Positive	Negative	4.0	4.5
	5	20	Positive	Negative	2.5	2.5
Case 4, H.K.	Susceptible	20	Positive	Negative	5.0	2.0
	—	—	—	—	—	—
	20	10	Positive	Negative	—	1.0
Case 8, H.Á.	5	—	—	—	—	—
	5	10	Positive	Positive	5.0	4.0
	10	—	Positive	—	5.0	1.0
Case 11, T.I.	10	80	Positive	Negative	2.0	1.0
	10	40	Positive	Negative	3.0	—
	10	10	Negative	Negative	1.0	—

In addition, these patients were clinically well and had no complaints during the observation period.

The results in patients in whom evidence of progression of the disease (bronchogenic spread, increase in the size of the cavity) was found roentgenographically during the observation period are shown in table 5. The patients who died are indicated in table 6.

TABLE 6

DEATHS

	Isoniazid Resistance		Catalase Activity		Virulence	
	Medium Without Isoniazid	Medium With Isoniazid	Medium Without Isoniazid	Medium With Isoniazid	Medium Without Isoniazid	Medium With Isoniazid
Case 3, P.J.	20	20	Positive	Negative	2.5	1.5
	40	10	Positive	Negative	3.5	2.0
	20	20	Positive	Negative	2.0	1.0
Case 9, K.M.	Susceptible	40	—	Positive	4.5	5.0
	10	10	Positive	Positive	4.5	1.0
	5	—	Positive	—	5.0	—
Case 10, P.I.	—	—	—	—	—	—
	20	20	Positive	Positive	5.0	4.0
	—	—	—	—	—	—

On the basis of the data shown in tables 4–6, the clinical findings for the patients in the present study were as follows: Six of the 13 patients observed did well clinically, 4 had progressive disease, and 3 died. In all of the three clinical groups, an isoniazid-susceptible strain was found in one instance. No significant difference was found in the level of isoniazid resistance of the three clinical groups.

By contrast, a very remarkable difference was found in the results of catalase-activity tests between the individual clinical groups. It was found that patients in good clinical condition excreted catalase-positive strains in 5 instances, and catalase-negative strains in 23 instances. Patients with progressive disease excreted catalase-positive strains in 11 instances, and catalase-negative strains in 8 instances.

From these data, it is apparent that the patients in good clinical condition yielded preponderantly catalase-negative strains. In contrast, catalase-positive strains predominated in patients who had progressive disease or who died. In this connection, it should be noted that 2 patients (*Cases 2* and *5*) in the "clinically well" group gave only catalase-negative strains in all of the tests.

Similar results were also obtained in the guinea pig virulence tests. Only one strain was found to be of lowered virulence in the group of deaths.

On the basis of the above data, it is evident that remarkable differences exist in the results of bacteriologic findings (catalase-activity, virulence tests) between the two types of clinical groups, namely the clinically well group, on the one hand, and the groups of progressive disease or death, on the other hand.

Six patients were subjected to pulmonary resection during the observation period. All of them were "poor-risk" patients with grave conditions clinically, and operation was undertaken as a desperate measure. This is well evidenced by the fact that in 3 of the 6 patients the operation performed was a second resection.

The results in patients in whom resectional operation was performed are shown in table 7. Prior to surgery, all of these patients had strains of tubercle bacilli susceptible to some of the major antituberculous agents, and these drugs were then given to them during the surgical period. Two of the patients who had

TABLE 7

BACTERIOLOGIC AND CLINICAL DATA OF PATIENTS OPERATED UPON WITH PULMONARY RESECTION

Extent of Resection	Isoniazid Resistance		Catalase Activity		Virulence		Clinical Condition
	Without Isoniazid	With Isoniazid	Without Isoniazid	With Isoniazid	Without Isoniazid	With Isoniazid	
Case 6, L.J.	20	20	Negative	Negative	3.5	3.0	In good clinical condition; sputum positive for tubercle bacilli
lobectomy	10	40	Positive	Negative	—	1.0	
	20	20	Negative	Negative	1.0	—	
Case 12,	—	—	—	—	—	—	In good clinical condition
U.J., resection; pulmonectomy	5	10	Negative	Negative	1.0	2.5	
	—	—	—	—	—	—	
Case 9,	Susceptible	40	—	Positive	4.5	5.0	
K.M., resection; pulmonectomy	10	10	Positive	Positive	4.5	1.0	Died
	5	—	Positive	—	5.0	—	
Case 10,	—	—	—	—	—	—	
P.I., pulmonectomy	20	20	Positive	Positive	5.0	4.0	Died
	—	—	—	—	—	—	
Case 11,	10	80	Positive	Negative	2.0	1.0	Bronchial fistula; empyema; progression on the contralateral side
T.I. resection; pulmonectomy	10	40	Positive	Negative	3.0	—	
	10	10	Negative	Negative	1.0	—	
Case 3,	20	20	Positive	Negative	2.5	1.5	
P.J., pulmonectomy	40	10	Positive	Negative	3.5	2.0	Died
	20	20	Positive	Negative	2.0	1.0	

resections and were in good clinical condition excreted preponderantly catalase-negative attenuated strains, while catalase-positive virulent strains predominated in patients with progressive disease or in those who died.

On the basis of the evidence obtained from the comparison of clinical and bacteriologic findings, the results can be summarized.

In general, it was found that there was some degree of relationship between bacteriologic findings and the clinical course of the patient. Catalase-negative attenuated strains were found primarily in those cases in which the clinical course of the disease proved to be favorable. This observation points to the fact already emphasized by other workers, principally by Middlebrook (*1d*), that the degree of catalase activity of the tubercle bacilli excreted by patients treated with isoniazid is correlated with the clinical course of the disease in most of the cases.

It is a point to be stressed, however, that the bacterial populations in strains isolated from tuberculous patients on isoniazid therapy cannot be regarded as

biologically homogenous. Great variations were found in the composition of such strains, and it was not unusual to find catalase-positive strains in the sputum of patients who previously excreted catalase-negative attenuated strains. This observation points to the fact that a single catalase-activity or virulence test is not sufficient for conclusions to be drawn with respect to the clinical course of the patient. It seems not unreasonable, however, to assume that serial catalase and virulence tests may prove of help in evaluating the clinical state of the patient. In all probability, the patients who excrete preponderantly catalase-negative strains will have a more favorable clinical course. Moreover, the small number of virulent but isoniazid-susceptible bacilli within such strains will be inhibited by the further administration of isoniazid.

In the light of our present knowledge, it should be emphasized that catalase-negative attenuated strains should not be considered harmless for man. Such strains still constitute a considerable danger to both the patient and public health. In addition, the significance for man of the decreased guinea pig virulence of isoniazid-resistant strains appears to be far from clear. In this connection, a fact which needs to be considered is the extreme impairment of the defensive mechanisms of the body in patients suffering from chronic, far advanced tuberculosis, a state which may lead to the fatal course of the disease even in the presence of attenuated strains.

The present writers agree with the views of Rist (7*b*) and Trendelenburg (8), which were supported and earlier expressed by Oestreicher (6), who stressed that all possible therapeutic measures should be resorted to in order to control the tuberculous process in the patients; and that attempts at producing isoniazid-resistant, catalase-negative, attenuated strains in the bodies of these persons by the prolonged administration of isoniazid may be justified only for those patients in whom all of the therapeutic measures have already failed. It is believed that patients excreting such strains should still be regarded as potential sources of infection. They need, therefore, to be isolated and their contacts actively immunized with BCG.

Summary

Strains of tubercle bacilli isolated from the sputum of 20 patients with moderately and far advanced cavitary tuberculosis have been regularly tested for virulence for the guinea pig, resistance to isoniazid, catalase activity, and cytochemical reactions. In carrying out these tests, strains cultured in isoniazid-containing medium and in isoniazid-free medium, respectively, were used. The results were:

A correlation could be demonstrated between catalase activity, resistance to isoniazid, and virulence for the guinea pig of tubercle bacilli.

No correlation could be demonstrated between the above tests and cytochemical reactions.

Sputum strains excreted by tuberculous patients cannot be regarded as biologically homogenous: great variations were found in the composition of the bacterial populations of these strains.

Patients excreting catalase-negative, guinea pig-avirulent strains were found to have favorable clinical courses during one year of observation.

Patients excreting catalase-positive, guinea pig-virulent strains were found to show progression of the disease or death.

Sumario

Importancia Bacteriológica y Clínica de la Actividad en Catalasa del Mycobacterium Tuberculosis

Cepas de bacilos tuberculosos aisladas del esputo de 20 enfermos que tenían tuberculosis cavitaria moderadamente o muy avanzada fueron comprobadas con regularidad en cuanto a virulencia para el cobayo, resistencia a la isoniacida, actividad catalásica, y reacciones citoquímicas. Al llevar a cabo eas pruebas, se usaron cepas cultivadas en medio con y sin isoniacida, respectivamente. Los resultados fueron:

Pudo descubrirse correlación entre la actividad catalásica, la resistencia a la isoniacida, y la virulencia para el cobayo.

No pudo descubrirse correlación entre las pruebas anteriores y las reacciones citoquímicas.

No cabe considerar como homogéneas biológicamente las cepas excretadas en el esputo por los tuberculosos: se descubrieron grandes variaciones en la composición de las poblaciones bacterianas de dichas cepas.

Los enfermos que excretaban cepas catalaso-negativas, avirulentas para el cobayo mostraron una evolución clínica favorable durante un año de observación.

Los enfermos que excretaron cepas catalaso-positivas, virulentas para el cobayo mostraron agravación de la enfermedad o fallecieron.

Resume

Signification bactériologique et clinique de l'activité catalase du M. tuberculosis

Des souches de bacilles tuberculeux, isolées de crachats chez 20 malades atteints de tuberculose cavitaire modérée ou très avancée, ont été régulièrement testées à l'égard de la virulence pour le cobaye, la résistance à l'isoniazide, l'activité de la catalase, et les réactions cytochimiques. Pour l'exécution de ces tests, on a utilisé respectivement les souches cultivées en milieu contenant de l'isoniazide ou en milieu dépourvu d'isoniazide. Les résultats furent les suivants:

Une corrélation entre l'activité de la catalase, la résistance à l'isoniazide et la virulence des bacilles tuberculeux pour le cobaye a pu être démontrée.

Aucune corrélation n'a pu être démontrée entre les tests ci-dessus et les réactions cytochimiques.

Les souches excrétées dans l'expectoration des tuberculeux ne peuvent être considérées homogènes du point de vue biologique: de grandes variations ont été découvertes dans la population bactérienne de ces souches.

Chez les malades excrétant des souches catalase-négatives et avirulentes pour le cobaye, l'évolution clinique s'est montrée favorable durant une année d'observation.

Chez les malades excrétant des souches catalase-positives et virulentes pour le cobaye, on a observé la progression de la maladie ou la mort.

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CLINICAL USE OF A SINGLE DAILY DOSE OF PARA-AMINOSALICYLIC ACID IN ASSOCIATION WITH ISONIAZID[1,2]

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INTRODUCTION

Although para-aminosalicylic acid (PAS) has been used in the treatment of tuberculosis for more than ten years, the optimal dose and regimen of therapy have not yet been determined. The usually recommended dosage is 3 gm. of PAS three or four times a day. As the free acid irritates the gastrointestinal tract, various salts or enteric preparations have been used and the dose has been adjusted as indicated. In spite of the use of these compounds, gastrointestinal irritation is common, and many patients are not able to take the recommended dose.

Experimental studies have shown that a single daily dose of PAS is adequate in the treatment of tuberculous guinea pigs whether the drug is given parenterally (1) or orally (2).

Previous clinical studies (3, 4) have demonstrated that a single daily dose of PAS is tolerated better than multiple doses and probably is therapeutically effective. The following study was carried out to learn more about the efficacy of a single daily dose of the drug, particularly when given with isoniazid.

MATERIALS AND METHODS

A series of 70 patients with tuberculosis were admitted to the study, which was conducted at Mineral Springs Sanatorium, Cannon Falls, Minnesota. Rest was prescribed according to symptoms, none of the patients being confined to bed all of the time. Para-aminosalicylic acid was given as tablets of the sodium or calcium salt, the dosage being 7 gm. (5 gm. of PAS) each morning after breakfast. The conventional dosage of isoniazid, 150 mg. twice a day, was given also.

Specimens of sputum or gastric washings were cultured for acid-fast bacilli before treatment and at monthly intervals during treatment. The susceptibility of all positive cultures to PAS and to isoniazid was determined in tubes containing 1, 4, and 16 γ of PAS per ml. of culture medium and 1 and 5 γ of isoniazid per ml. of culture medium. For this study, drug resistance was defined as growth in the tube containing 1 γ of the drug equal to the growth in the control tube.

Roentgenograms of the chest were made before treatment and after one, two, and four months of treatment.

The four months of therapy and the bacteriologic studies necessary for analysis of therapeutic results were completed in 47 cases. Others were excluded for various reasons such as exclusively extrapulmonary location of the disease, resistance of the tubercle bacilli to PAS or isoniazid before treatment, or premature departure from the hospital. Only 3 cases had to be excluded because of the patients' inability to take the recommended dose of PAS.

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[2] Presented at the Veterans Administration-Armed Forces Conference on Chemotherapy of Tuberculosis, Memphis, Tennessee, February 3 to 6, 1958.

In the 47 cases retained, 23 of the patients were males whose average age was fifty-six years, and 24 were females whose average age was forty-one years. Forty patients had had no previous antituberculous chemotherapy, and 7 had had such treatment previously. Tubercle bacilli were recovered from all 47 patients, and the virulence of the bacilli was proved in each case by inoculation of guinea pigs. In extent, the disease was minimal in 11 patients, moderately advanced in 22, and far advanced in the remaining 14.

Results

As mentioned, only 3 patients were unable to take the recommended dose of PAS. A few others had some gastrointestinal irritation but were able to continue treatment with the same or another salt of para-aminosalicylic acid. Three patients switched to Rezipas®, with satisfactory results. An allergic reaction occurred in 2 cases, requiring desensitization to PAS. There were no untoward reactions to isoniazid.

The small number of reactions with the single daily dose of PAS is in sharp contrast to experience with the conventional dosage. In another study, among a series of 192 patients at Mineral Springs Sanatorium given 3 gm. of PAS (as tablets of the sodium or the calcium salts) three times daily, 31 had to stop taking the drug because of gastrointestinal irritation (table 1).

The results of bacteriologic studies among the series of 47 patients are summarized in table 2. Although tubercle bacilli had been recovered from all patients before treatment, after two months of treatment tubercle bacilli were recovered from only 9 (19 per cent). After four months of treatment only 5 (11 per cent) had cultures positive for tubercle bacilli, a conversion rate of 89 per cent.

TABLE 1

Intolerance to PAS in Association with Isoniazid

Dose of PAS	Number of Patients Treated	Number of Patients Intolerant	Per Cent of Patients Intolerant
5 gm. once daily	70	3	4
3 gm. thrice daily	192	31	16

TABLE 2

Single Daily Dose of PAS in Association with Isoniazid: Bacteriologic Studies

Extent of Disease	Number of Patients Treated	Number of Patients with Cultures Negative for Tubercle Bacilli	
		After 60 days	After 120 days
Minimal	11	11	11
Moderately advanced	22	19	21
Far advanced	14	8	10
Total	47	38	42

TABLE 3

SINGLE DAILY DOSE OF PAS WITH TWICE-DAILY DOSE OF ISONIAZID: INCIDENCE OF DRUG RESISTANCE

Period of Treatment	Number of Cultures Positive for Tubercle Bacilli	Number of Cultures Susceptible to Isoniazid and PAS	Number of Cultures Resistant to	
			Isoniazid	PAS
days				
60	9	7	1	2
120	5	2	3	2

TABLE 4

SINGLE DAILY DOSE OF PAS IN ASSOCIATION WITH ISONIAZID: ROENTGENOGRAPHIC STUDIES AFTER 120 DAYS OF TREATMENT

Extent of Disease	Number of Patients Treated	No Change	Improvement	
			Minimal	Moderate or Marked
Minimal	11	3	0	8
Moderately advanced	22	5	4	13
Far advanced	14	6	4	4
Total	47	14	8	25

The results of the drug-susceptibility studies are summarized in table 3. After 120 days of treatment, tubercle bacilli susceptible to both PAS and isoniazid were recovered from 2 patients. Treatment was continued, and cultures from these 2 patients are now negative for tubercle bacilli. Drug-resistant bacilli were recovered from 3 patients. The microorganisms were resistant to 5 γ of isoniazid per ml. of culture medium in 2 cases and to 1 γ in the third. Two of these same cultures were resistant to 1 γ of PAS per ml. of culture medium.

The results of comparisons of the chest roentgenograms are summarized in table 4. Many of the patients had persistent cavities, and some have undergone resection. In others, age, general condition, and extent of disease have made surgical treatment seem unwise, so chemotherapy has been continued.

COMMENT

The small number of the available patients precluded employment of a concomitant control series. However, the results obtained with the regimen employed should be compared with those observed after treatment with the conventional dosage of PAS plus isoniazid. At the Sixteenth Conference on the Chemotherapy of Tuberculosis, Bowerman (5) reported that, after sixteen weeks of treatment with isoniazid (3 to 5 mg. per kg. of body weight per day) and PAS (10 to 12 gm. per day in divided doses), cultures negative for tubercle bacilli were obtained from 85 per cent of 231 cases. He also reported that treatment with the same dose of isoniazid along with 5 to 6 gm. of PAS per day in divided doses for sixteen weeks resulted in negative cultures in only 68 per cent of 233 cases.

With the assumption that the groups of patients were comparable, it seems that a single daily dose of 5 gm. of PAS is as good as 10 to 12 gm. per day in divided doses and is superior to 5 to 6 gm. of PAS per day in divided doses.

Summary

In a series of 47 cases of pulmonary tuberculosis, a single daily dose of 5 gm. of PAS (7 gm. of the sodium or calcium salts) given in association with 150 mg of isoniazid twice daily was both better tolerated and as equally effective as the conventional dosage of 3 gm. of PAS given three or four times a day. The advantages of less gastrointestinal irritation and economy suggest that this regimen should be studied more extensively.

Sumario

Empleo Clínico de una Sola Dosis Diaria de PAS Combinado con Isoniacida

En una serie de 47 casos de tuberculosis pulmonar, una sola dosis diaria de 5 gm. de PAS (7 gm. de la sal sódica o la cálcica), administrada junto con 150 mg. de isoniacida dos veces diarias fué a la vez mejor tolerada e igualmente eficaz que la dosis aceptada de 3 gm. de PAS administrada tres o cuatro veces al día. Las ventajas de la menor irritación intestinal y de la economía sugieren un estudio más extenso de este régimen.

Resume

Emploi clinique d'une dose unique quotidienne de PAS associée à l'isoniazide

Dans une série de 47 cas de tuberculose pulmonaire, une seule dose quotidienne de 5 g de PAS (7 g du sel de calcium ou du sel de sodium), associée à 150 mg d'isoniazide deux fois par jour, était mieux tolérée et aussi efficace que la dose classique de 3 g de PAS administrée trois ou quatre fois par jour. Les avantages offerts par une moindre irritation gastro-intestinale et une moindre dépense suggèrent une étude plus ample de ce régime thérapeutique.

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EFFECT OF SINGLE AND OF DOUBLE DAILY DOSES OF PARA-AMINOSALICYLIC ACID IN TUBERCULOSIS OF GUINEA PIGS[1,2]

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INTRODUCTION

Early experimental work in tuberculosis of guinea pigs showed that, when para-aminosalicylic acid (PAS) was given mixed with the feed, a high daily intake of the drug and prolonged treatment were necessary in order to achieve marked therapeutic effect. For example, in an exploratory experiment in which tuberculous guinea pigs were given a diet containing 2 per cent PAS for forty-eight days there was no significant beneficial effect. In contrast, when the concentration of PAS in the feed was 4 per cent and treatment was continued for 119 days, the deterrent effect on the disease was impressive. The animals each ate an average of 40 gm. of feed a day, which provided an intake of approximately 1,600 mg. of PAS spread over a twenty-four hour period (1). Subsequent studies in this laboratory indicated that the administration of 4 per cent PAS in the diet to tuberculous guinea pigs had to be continued for a minimum of sixty days to produce any significant therapeutic effect.

When studies were started on the effect of the sodium salt of PAS (NaPAS) given subcutaneously to tuberculous guinea pigs, it was found that much less drug was needed daily than when given in the feed. In a preliminary experiment in which treatment was started twenty-one days after infection, it was found that a single daily subcutaneous injection of 125 mg. of NaPAS was highly beneficial when given for only fifty days. Furthermore, it was found that single daily injections of 250 and of 500 mg. of NaPAS were of no greater value than the 125-mg. dose (2).

In the following account two experiments are described which show that single daily doses of NaPAS administered either subcutaneously or orally result in a pronounced therapeutic effect, and also that no advantage appears from giving the drug twice daily either in divided amounts or in two full doses.

OBSERVATIONS

Experiment 1

Forty mature male guinea pigs, each weighing approximately 600 gm., were each infected subcutaneously over the sternum with 0.1 mg. (moist weight) of tubercle bacilli (H37Rv). On the twenty-first day of infection the animals were divided into groups con-

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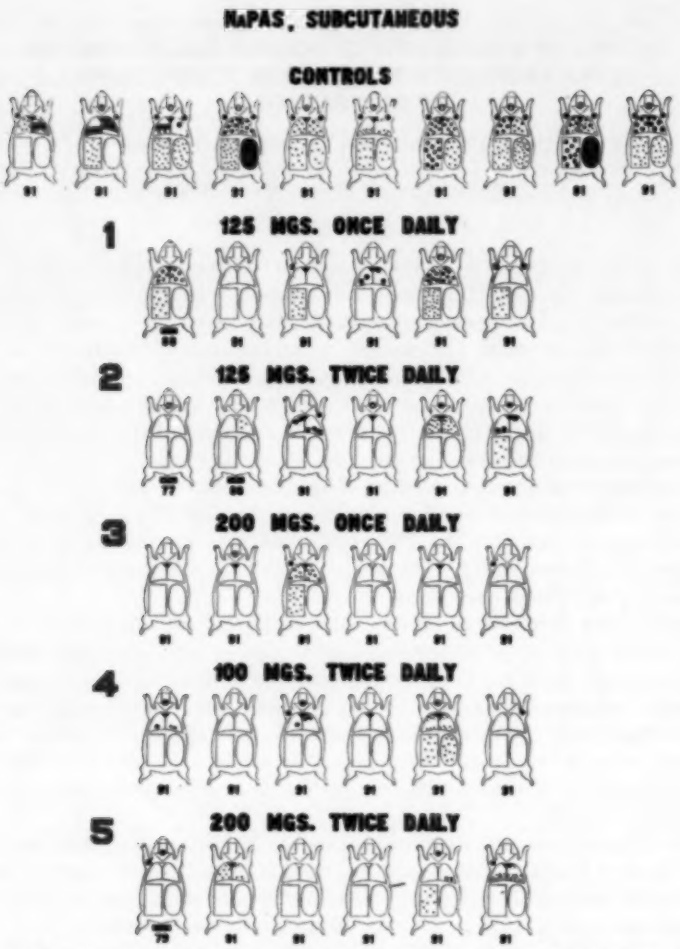

FIG. 1. (*Experiment 1*). Schematic record of the extent of tuberculous lesions seen at necropsy. The rectangle and oval represent liver and spleen, respectively. The arrow represents the site where bacilli were injected. Dots represent miliary and nodular lesions, and blackening indicates diffuse involvement. The bar under a figure indicates that the animal died before the end of the experiment. The small numerals indicate the number of days of infection. Treatment started on the twenty-first day of infection. The amount of NaPAS indicated for each group was given subcutaneously in 1 ml. of solution. The doses given twice daily were spaced at twelve-hour intervals. All surviving animals were killed on the seventieth day of treatment.

sisting of one control group of 10 and five other groups of 6 animals each to be treated as shown in figure 1. The required amount of NaPAS in 1 ml. of solution was injected subcutaneously in the axillary space. Treatment was administered seven days a week for ten weeks. For the animals treated twice daily, the injections were given at twelve-hour inter-

vals.[3] After seventy days of treatment the animals were killed and a record was made of the lesions seen at necropsy.

In figure 1 it may be seen that among the untreated controls there was extensive tuberculous disease in the usual organs of predilection. Comparison of groups 1 and 2 indicates that little was gained by giving doses of 125 mg. of NaPAS twice daily as opposed to giving 125 mg. once daily. Comparison of groups 3, 4, and 5 shows that a single injection of 200 mg. of NaPAS was as effective as 100 mg. given twice daily; and in addition, giving 200 mg. once daily appeared to be as beneficial as giving this amount twice daily.

Histopathologic examination was made of lung, liver, and spleen from each animal. In contrast to the extensive tuberculous lesions seen in tissues from the control animals, histologic evidence of retardation of the disease, such as fibrosis and residual foci of epithelial cells with little or no necrosis, was found in the treated animals. There was no significant difference among the five treated groups.

The extent and character of the lesions as seen microscopically were recorded numerically as an index of infection (3). The average index of infection for each group was as follows: controls, 87; group 1, 29; group 2, 22; group 3, 27; group 4, 28; and group 5, 28.

Experiment 2

In the second study the NaPAS was given orally by means of a stomach tube. Thirty-eight mature male guinea pigs, each approximately 500 gm. in weight, were each inoculated intraperitoneally with 0.1 mg. (moist weight) of tubercle bacilli (H37Rv). On the twentieth day of infection 6 animals were killed as pretreatment controls and were found to have evidence of extensive tuberculous disease, as shown in figure 2. Also on the twentieth day of infection, the remaining 32 animals were divided into four groups of 8 animals each. One group served as controls and the other three were treated as shown in figure 2.

The NaPAS was given in 1 ml. of solution. In the group treated twice daily, the doses were given eight hours apart. The daily amount of 200 mg. of NaPAS was selected because of previous experience which showed that a single daily oral dose of 100 mg. of NaPAS given for sixty days produced only a partial—although definite—beneficial effect on tuberculosis in guinea pigs (4). A schedule of treatment on Monday, Wednesday, and Friday of each week was selected for two of the groups because it had been found in study of isoniazid that a single daily oral dose of 10 mg. of that drug given each Monday, Wednesday, and Friday for a sixty-day period has a therapeutic effect equivalent to that of daily treatment with the same dose (5).

After sixty-six days of treatment all surviving animals were killed. The extent of tuberculous lesions was recorded schematically, as shown in figure 2.

As indicated for the pretreatment controls in figure 2, the disease was in an advanced state when treatment was started, thus providing a formidable process for a chemotherapeutic test. This is reflected in the early deaths and extensive disease seen in the controls.

Among those treated with 200 mg. of NaPAS once daily, one animal died in thirty-nine days (after nineteen days of treatment), and that instance is considered a failure. It is apparent that a single daily oral amount of 200 mg. of

[3] The NaPAS used in this and in the second experiment was furnished through the courtesy of Parke, Davis and Company, Detroit, Michigan.

FIG. 2 (*Experiment 2*). Schematic record of the extent of tuberculous lesions seen at necropsy (*see* figure 1 for explanation of drawings). Treatment was started on the twentieth day of infection and was continued for sixty-six days. The doses of NaPAS were given via stomach tube in 1 ml. of solution. The doses given twice daily were given eight hours apart.

NaPAS for sixty-six days resulted in pronounced regression and disappearance of the lesions presumed to have been present when treatment started. The 2 animals which died from unknown causes after only fifty-three and fifty-six days of treatment, respectively, had benefited thus.

Among the groups treated only on Monday, Wednesday, and Friday it was seen that, in comparison to eventualities in the control animals, some thera-

peutic effect was achieved; but the result was inferior to that produced by daily administration. In addition, it appeared that no benefit was derived from dividing the 200-mg. amount into two 100-mg. doses given eight hours apart.

Histopathologic studies on the liver, spleen, and lungs of animals in this experiment confirmed the necropsy findings. An average index of infection, based on the histologic character of the lesions, was determined for each group as follows: controls, 95; group given 200 mg. once daily (excluding the failure), 1.62; group given 200 mg. once each Monday, Wednesday, and Friday, 31.5; group given 100 mg. twice each Monday, Wednesday, and Friday, 33.0.

Serum Concentrations of PAS

Determinations of the concentration of PAS in the blood were not made in the studies reported above. However, certain information is available which suggests that in guinea pigs a single oral dose of NaPAS is followed by an initial high blood concentration which declines rapidly in a twelve-hour period. In normal guinea pigs given a single oral dose of 400 mg. of NaPAS, serum concentrations were determined as follows in mg. per 100 ml.: one hour, 5.5 to 6.2; two hours, 1.9 to 2.1; eight hours, 0.6 to 0.9 (4). In contrast, concentrations in the blood of infected animals that were given a diet containing 4 per cent PAS for 119 days (and were estimated to have consumed approximately 1,600 mg. of PAS in each twenty-four-hour period) were found to be less than 0.5 mg. per 100 ml. (1).

The *in vitro* tuberculostatic effect of serum from guinea pigs at intervals following a single oral dose of 200 mg. of NaPAS has been determined. Each of eight 500-gram guinea pigs was given 200 mg. of NaPAS in 1 ml. of solution. Two animals were then bled by cardiac puncture at two, four and one half, eleven, and twenty-four hours, respectively. Serial twofold dilutions of serum from 1:4 up to 1:2,048 were made in 2-ml. amounts of Proskauer and Beck liquid medium containing 5 per cent horse serum. Each tube containing 2 ml. of diluted serum was inoculated with 0.01 mg. of tubercle bacilli (H37Rv). After fourteen days of incubation, the greatest dilution that inhibited growth was recorded as the tuberculostatic dilution as follows: two hours, 1:1,024; four and one half hours, 1:4; eleven hours, 1:4 and less; and twenty-four hours, 1: <4.

Comment

The observations reported here indicate that, to achieve therapeutic effect in tuberculosis of guinea pigs, PAS need not be given "around the clock," as when administered with the feed, or even in multiple daily doses. A single daily dose, either parenteral or oral, is sufficient if given for approximately sixty days. The limited studies of concentration in the blood suggest that in guinea pigs it may not be necessary to maintain a constant blood concentration of PAS.

In a study of streptomycin it was found that against tuberculosis of guinea pigs a single daily dose was as effective therapeutically as four doses daily (6). In view of the rapid excretion of streptomycin by guinea pigs, it was postulated that either a sufficient amount of the antimicrobial adheres to the tissues or to bacterial cells or that a single daily and brief exposure to streptomycin may be

sufficient to arrest the infection. In the case of PAS, either or both factors may be operative.

Summary

In an experiment on the subcutaneous administration of the sodium salt of PAS (NaPAS) to tuberculous guinea pigs, treatment was started on the twenty-first day of infection and was continued for seventy days. It was found that a single daily dose of 125 mg. of NaPAS was as effective as two such daily doses. Also, it was found that a single daily dose of 200 mg. of NaPAS was as effective as two 100-mg. daily doses. The single 200-mg. daily dose was as effective as two 200-mg. daily doses.

In a second experiment, in which treatment of tuberculous guinea pigs was started on the twentieth day of infection and continued for sixty-six days, it was found that a single oral daily dose of 200 mg. of NaPAS was a markedly effective regimen. Administration of a single 200-mg. dose orally each Monday, Wednesday, and Friday for ten weeks was less effective than the daily regimen, and dividing the daily amount to provide two 100-mg. doses each Monday, Wednesday, and Friday appeared to offer no advantage.

Sumario

El Efecto de las Dosis Diarias Unicas y Dobles de PAS en la Tuberculosis del Cobayo

En un experimento relativo a la administración subcutánea de la sal sódica del PAS (NaPAS) a cobayos tuberculosos, se inició el tratamiento al vigésimoprimer día de la infección, continuándose setenta días. Observóse que una dosis diaria única de 125 mg. de NaPAS era tan eficaz como dos dosis diarias de igual tamaño. Además, se observó que una dosis diaria única de 200 mg. de NaPAS era tan eficaz como dos dosis diarias de 100 mg. La dosis diaria única de 200 mg. resultó tan eficaz como dos dosis diarias de 200 mg.

En otro experimento, en el cual se inició el tratamiento de los cobayos al vigésimo día de la infección y se continuó sesenta y seis días, se descubrió que una sola dosis diaria oral de 200 mg. de NaPAS constituía un régimen notablemente eficaz. La administración de una sola dosis de 200 mg. oralmente los lunes, miércoles y viernes durante diez semanas fué menos eficaz que el régimen diario y al parecer no se ganó nada con la fraccionación de la cantidad diaria para suministrar dos dosis de 100 mg. los lunes, miércoles y viernes.

Resume

Effet des doses simples et doubles de PAS dans la tuberculose du cobaye

Dans une expérimentation de l'administration sous-cutanée du sel sodique du PAS (NaPAS) à des cobayes tuberculeux, le traitement a été commencé au vingt-et-unième jour de l'infection et poursuivi durant soixante-dix jours. Il a été observé qu'une dose unique quotidienne de 125 mg de NaPAS était aussi efficace que deux doses quotidiennes semblables. On a également constaté qu'une dose unique quotidienne de 200 mg de NaPAS était aussi efficace que deux doses de 100 mg par jour. La dose unique quotidienne de 200 mg par jour était aussi efficace que deux doses de 200 mg par jour.

Au cours d'une seconde expérimentation dans laquelle le traitement des cobayes tuberculeux avait débuté au vingtième jour de l'infection et était poursuivi pendant soixante-six jours, il a été observé qu'une dose unique de 200 mg par jour par voie orale était une posologie d'efficacité marquée. L'administration d'une dose orale de 200 mg chaque lundi, mercredi et vendredi pendant dix semaines était moins efficace que l'administration quoti-

dienne. La répartition de la dose quotidienne en deux doses de 100 mg chaque lundi, mercredi et vendredi n'a pas paru offrir d'avantages particuliers.

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Case Reports

COMBINED CRYPTOCOCCAL AND TUBERCULOUS MENINGITIS COMPLICATING RETICULUM CELL SARCOMA[1]

BENJAMIN BURROWS AND WILLIAM R. BARCLAY

(Received for publication July 9, 1958)

INTRODUCTION

The increased incidence of tuberculous and fungal infection in patients with malignant lymphomas is now generally recognized. Despite reports of simultaneous systemic involvement with *M. tuberculosis* and *C. neoformans* (1, 2), combined meningeal infection has not been previously noted in such patients. Only a single case of coexistent tuberculous and cryptococcal meningitis has been reported, and this in a 10-year-old child, without evidence of malignancy of the reticuloendothelial system. In reporting this case, Nanda and associates (3) cite the unpublished observations of Christie who has apparently encountered several such instances of combined meningitis.

The patient whose case is reported here developed meningitis while on isoniazid therapy for pulmonary tuberculosis complicating a reticulum cell sarcoma. Both *M. tuberculosis* and *C. neoformans* were isolated from the cerebrospinal fluid ante mortem. To the writers' knowledge, this is the second case of combined cryptococcal and tuberculous meningitis to be reported, and the first in association with a lymphoma.

CASE REPORT

A 46-year-old white housewife was admitted to the University of Chicago Clinics in June, 1951, complaining of masses over the occipital region of the skull for the previous three months. Her history dated from 1945 when a diagnosis of "lymphoblastoma" was made at another hospital on biopsy of an inguinal mass. Between 1945 and 1951, courses of radiation therapy were administered to the inguinal region, upper abdomen, axilla, and posterior cervical nodes, with prompt disappearance of tumors in these areas.

The past history revealed a six-month hospitalization for active pulmonary tuberculosis in 1934. A right phrenicotomy was performed at that time, and the patient apparently made a satisfactory recovery without evidence of reactivation of the pulmonary disease.

Physical examination at the time of her initial referral in 1951 revealed a well-nourished, well-developed woman in no apparent distress. Positive findings included two masses in the scalp over the occipital region and small nodes in the left supraclavicular area and in the left axilla. The right diaphragm was elevated and immobile. A left upper quadrant abdominal mass was noted and interpreted as spleen, although its configuration was atypical. Mild edema of the right leg was found, apparently dating from a course of X-ray therapy to the inguinal nodes on that side.

Laboratory studies showed a negative serologic test for syphilis; leukocytes, 3,300 per cu. mm.; hemoglobin, 11.6 gm.; erythrocytes, 3.69 million. Differential smear revealed 79 per cent polymorphonuclears, 16 per cent lymphocytes, 2 per cent monocytes, 3 per cent eosinophils, no basophils, and no abnormal cells. Urinalysis was unremarkable. The initial chest film (figure 1) showed fibrocalcific lesions in the right upper lung field, considered to be inactive residua of old tuberculosis. There was deviation of the trachea to the right and elevation of the right hemidiaphragm. Biopsy of a left axillary node revealed obliteration

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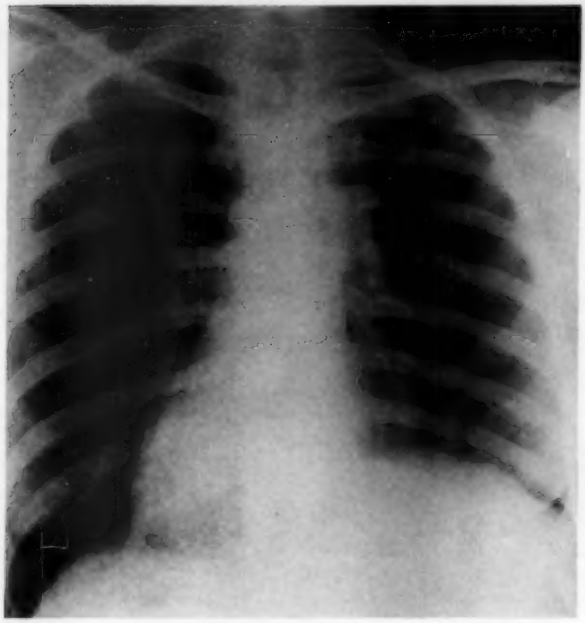

FIG. 1. Roentgenogram obtained in September, 1951, revealing fibrocalcific lesions in the right upper lung field.

of normal node structure by a homogeneous mass of cells having the histologic characteristics of a reticulum cell sarcoma.

Between 1951 and 1954, numerous courses of X-ray therapy were administered for masses in the scalp, abdomen, axilla, groin, and pre-auricular areas, always with prompt disappearance of the tumors. The patient's course was complicated by herpes zoster. In addition, pain, muscle contracture, and marked weakness of the right arm developed in 1952 and a course of X-ray therapy was directed to the cervical and upper thoracic vertebrae for apparent nerve-root compression at these sites. Lumbar puncture at that time revealed normal dynamics and completely normal cerebrospinal fluid. In 1953 there was an episode of acute pyelonephritis; an intravenous pyelogram was normal following this episode.

A repeat chest film in August, 1953, revealed no change in appearance of the fibrocalcific lesions; but, on another film in May, 1954, multiple nodular lesions were noted in the right upper lobe. A lesion was also noted in the left second rib, and there was widening of the upper mediastinum. Since a reactivation of tuberculosis or pulmonary spread of sarcoma could not be distinguished, no specific therapy was undertaken. By April, 1955, the pulmonary lesions had increased in size (figure 2). The patient was hospitalized, and three gastric lavages were obtained; these were negative for tubercle bacilli on culture. At the time of this admission, routine urinalysis revealed a trace of albumin, numerous leukocytes, and an occasional erythrocyte. Acid-fast bacilli could not be cultured from the urine, but a repeat intravenous pyelogram revealed the development of bilateral hydronephrosis. On retrograde pyelography, bilateral ureteral obstruction was demonstrated and the patient received X-ray therapy to the region of these obstructions.

No change in the nodular pulmonary lesions was noted in the several months following

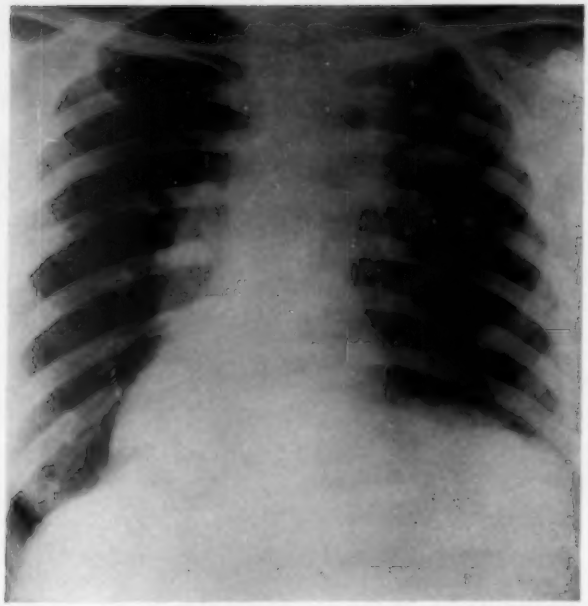

FIG. 2. Repeat roentgenogram obtained in April, 1955, showing the development of multiple nodules in the right upper lobe.

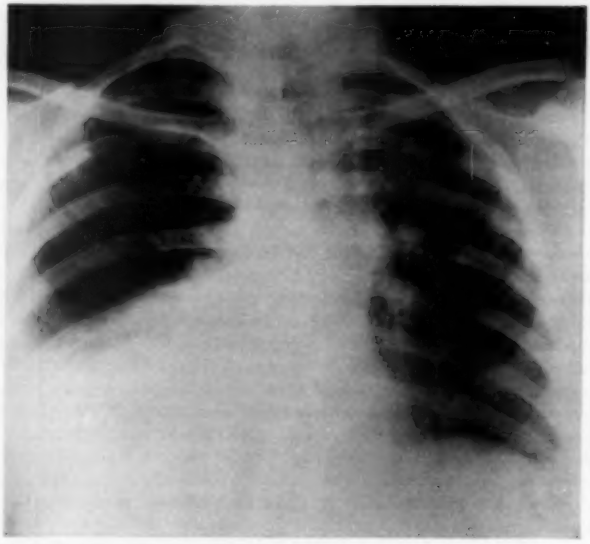

FIG. 3. Roentgenogram obtained in October, 1955, revealing cavitation in one of the pulmonary nodules. The sputum became positive on culture for tubercle bacilli at this time. A left pleural effusion was also noted.

the April, 1955, admission, but in October of that year a left pleural effusion developed. Neither malignant cells nor tubercle bacilli could be isolated from the pleural fluid. A few weeks later, a repeat chest film revealed cavitation of one of the pulmonary nodules and a persisting pleural effusion (figure 3); isoniazid, 100 mg. three times daily, was begun despite negative microscopic examination of the sputum for acid-fast bacilli. Later, these same sputum specimens were found to be positive for tubercle bacilli on culture. A few days after the onset of isoniazid therapy, the patient received a course of intravenous nitrogen mustard.

Tubercle bacilli were cultured from the sputum in December, 1955, and the patient was re-hospitalized. A chest film at that time revealed further cavitation of one of the nodules, with the development of a thin-walled, cystic-appearing lesion. The other nodules had almost completely disappeared, and the left pleural effusion had diminished in amount. Complete blood counts revealed no significant change from initial values. Platelets were 170,000 per cu. mm. Urinalysis was completely normal, and the blood urea nitrogen was 27.8 mg. per 100 ml. Isoniazid assay (microbiologic technique) revealed a concentration of approximately 0.5 γ per ml. in the blood following an oral dose of 100 mg. Repeated spu-

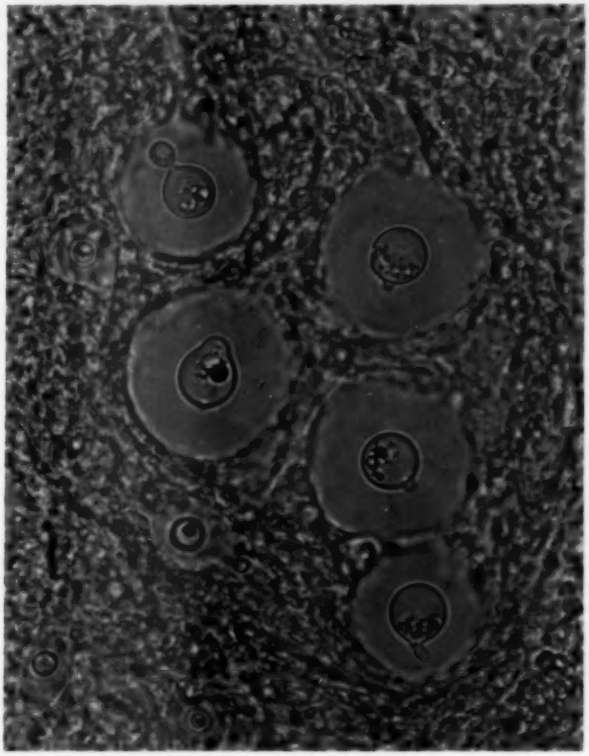

FIG. 4. Photomicrograph (1,300 ×) of a smear of mouse brain after inoculation of the mouse with cerebrospinal fluid from the patient. The smear has been impregnated with india ink and reveals typical *C. neoformans*.

tum examinations were negative on microscopy but positive on culture for *M. tuberculosis*. Para-aminosalicylic acid (PAS), 3 gm. four times daily, was added to the isoniazid regimen, and the patient was discharged from the hospital on these medications.

The pleural effusion continued to accumulate, requiring several therapeutic thoracenteses for dyspnea during the succeeding months. At the time of readmission in June, 1956, the sputum had become consistently negative for tubercle bacilli by microscopy and culture. There had been considerable clearing of the pulmonary infiltrate, with disappearance of the cavitary lesion. However, the pleural effusion persisted and the patient complained of severe nausea and gastrointestinal disturbance. Distrycin® therapy was substituted for PAS, with temporary relief of the gastrointestinal complaints. A few weeks later, the nausea returned, accompanied by extreme dizziness, clearly related in time to the streptomycin injections and requiring their discontinuance. Following this, antituberculous chemotherapy was limited to isoniazid, 150 mg. three times daily.

At about this time, the patient noted dysphagia, and a twelfth cranial nerve palsy with atrophy of the left side of the tongue was diagnosed. Over the next few months spontaneous improvement in the dysphagia was noted as well as objective improvement in mobility of the tongue.

In October, 1956, the patient noted the sudden onset of generalized headache, feverishness, irritability, anorexia, and progressive drowsiness. Temperature on admission was 39.2°C. and there was nuchal rigidity. Lumbar puncture revealed an initial pressure of 400 mm. of fluid. The cerebrospinal fluid was clear and colorless but revealed 198 cells per cu. mm., 178 of them mononuclears. The pandy was 1-plus; total proteins, 82 mg. per 100 ml.; sugar, 52 mg. per 100 ml. (blood sugar, 102); chlorides, 102 mm. per liter. Cryptococci were found on culture and were pathogenic for mice (figure 4). Cryptococci were found repeatedly on subsequent examinations, and *M. tuberculosis* was cultured from a specimen of cerebrospinal fluid obtained five days after admission. The patient was started on intravenous sulfadiazine, and the concentrations of drug were maintained between 7.5 and 13.6 mg. per 100 ml. of serum. Isoniazid was increased to 400 mg. twice daily and, because of the extreme toxicity, prednisone, 25 mg. per day, was added to the program. The patient's course was very stormy; she became stuporous during the first several days of hospitalization and developed a syndrome of dehydration, sodium depletion, and metabolic acidosis. On therapy, the electrolyte picture improved, and the temperature and mental status gradually returned to normal. The blood urea nitrogen showed a tendency to rise, reaching a high of 65 mg. per 100 ml. during this admission, and a repeat intravenous urogram disclosed nonvisualization on the right with marked left hydronephrosis. By the end of one month of hospitalization, there was improvement in the cerebrospinal fluid with 42 cells per cu. mm., all mononuclear; and the protein was 32 mg. per 100 ml. The cerebrospinal fluid sugar which had fallen to a low of 16 mg. per 100 ml. showed a rise to 58. Cryptococci were still easily isolated, but no tubercle bacilli could be cultured. Several blood transfusions were necessary to maintain the hemoglobin above 8 gm. per 100 ml. By the thirty-third hospital day, the patient was fully ambulant, afebrile, and relatively asymptomatic. She was then allowed to leave the hospital on continued isoniazid and sulfonamide therapy.

A few weeks following discharge from the hospital, there was a return of occipital headache, followed by nuchal rigidity, nausea, projectile vomiting, bilateral tinnitus, and intermittent fever. The patient was readmitted to the hospital. Cerebrospinal fluid revealed a pressure of 460 mm. of fluid, 170 cells per cu. mm. The pandy was 1-plus; the total protein, 88 mg. per 100 ml.; sugar, 4 mg. per 100 ml.; numerous cryptococci were revealed on direct examination. Pyuria and bacilluria were noted and responded to a course of tetracycline. A roentgenogram of the chest revealed no change in the fibrotic-appearing strands which had replaced the old nodular lesions in the left subapical region (figure 5). By the fifth hospital day, the patient's general condition had deteriorated, and a daily intravenous infusion of 50 mg. of amphotericin B was begun. Since no therapeutic effect was noted, this was increased to 100 mg. daily after a week of therapy. A daily temperature elevation as high as 39.5°C. followed each infusion. The cerebrospinal fluid revealed a fall

FIG. 5. Roentgenogram obtained in January, 1957, showing resolution of the pulmonary nodules and the pleural effusion.

in pressure to 160 mm., a fall in protein to 20 mg. per 100 ml., a rise in sugar to 62 mg. per 100 ml.; for the first time since the onset of meningitis, *C. neoformans* could not be demonstrated on microscopy or culture. However, the cell count had increased to 300 per cu. mm. Renal studies showed a progressive deterioration with blood urea nitrogen rising to 108 mg. per 100 ml. In addition, a bacteremia due to a drug-resistant strain of *E. coli* developed and failed to respond to chloramphenicol therapy. The patient became progressively more stuporous and finally died on February 19, 1957, twelve years after the original diagnosis of malignant lymphoma.

Post-mortem examination: Reticulum cell sarcoma was found involving the pelvic, periaortic, and periadrenal tissue and multiple groups of lymph nodes. There was partial obstruction of both pelvic ureters from sarcomatous tissue with bilateral hydro-ureteropyelonephrosis and severe hydronephrotic atrophy of the left kidney. Acute and chronic pyelonephritis was noted on the right. Sarcomatous involvement of the right brachial plexus was noted as well as invasion of the mediastinal fat and the esophagus. There was chronic congestive splenomegaly with fibrosis. Multiple scars and calcific nodules were noted in the right upper lobe of the lung without evidence of active tuberculous or cryptococcal infection. Several broncholiths were observed and there was mild, bilateral, cylindrical bronchiectasis. Obliterative fibrinous pleuritis was noted bilaterally. Meningitis and a moderate external hydrocephalus were observed. The leptomeninges were thickened and showed a mild mononuclear cell inflammation with giant cells in which *C. neoformans* could be identified. Organisms were also noted free in tissue, often in perivascular locations. No evidence of tuberculous meningitis was found. Other abnormalities noted on post-mortem examination included an acute esophagitis, secondary hemosiderosis of spleen and lymph nodes, anemia, subacute duodenal ulcer, Meckel's diverticulum, acute and chronic cystitis, stercoral ulcers in the rectum, atrophy of the thyroid gland, fatty change

in the liver, scarring of the left cornea, ossification and bone marrow formation in bronchial cartilages, and hyperplasia of the eosinophils in the pituitary gland.

Comments

Simultaneous isolation of *Cryptococcus neoformans* and *Mycobacterium tuberculosis* from the cerebrospinal fluid is a rarity and, in this case, was presumably related to an increased susceptibility to these organisms in association with a malignant lymphoma. The patient was known to have recovered from active pulmonary tuberculosis more than ten years before the onset of her reticulum cell sarcoma, and proof of reactivation of the pulmonary disease was obtained a year before the onset of meningitis. Single-drug therapy with isoniazid failed to prevent the appearance of *M. tuberculosis*, despite control of the pulmonary disease. The development of meningitis during isoniazid therapy has been reported (4), but is unusual. In addition to reduced resistance to tuberculous infection, the presence of an active meningitis from another cause may have facilitated the involvement of the meninges by *M. tuberculosis*. The source of tubercle bacilli in the cerebrospinal fluid remains obscure. There was never evidence of a hematogenous spread of the tuberculosis, nor at autopsy was a tuberculous lesion found adjacent to the meninges, which could account for the meningitis. It is improbable that nitrogen mustard therapy provoked the presence of bacilli in the cerebrospinal fluid since it was administered over one year prior to the appearance of the meningitis. From the clinical and laboratory findings, it seems probable that the meningitis was primarily due to Cryptococcus and that *M. tuberculosis* may have been an incidental invader. In this respect, *M. tuberculosis* could not be isolated from the cerebrospinal fluid after the dose of isoniazid was raised from 150 mg. three times daily to 400 mg. twice daily, but a lasting remission of the meningeal inflammation was not produced. Unfortunately, drug-susceptibility tests were not performed on the tubercle bacilli isolated from the cerebrospinal fluid. Nine months previously, organisms in the sputum were susceptible to 1 γ of isoniazid per ml. and resistant to 0.1 γ per ml. The development of relative drug resistance prior to the meningitis is certainly possible, although the sputum remained completely negative for *M. tuberculosis* by microscopy and culture. Whether involvement of the meninges by *M. tuberculosis* might have been prevented by administration of PAS or streptomycin in association with isoniazid cannot be determined. Single-drug therapy was used in this patient because of intolerance to the other medications.

The greatest diagnostic problem presented by this case was to determine the nature of the nodular subapical lesions. During the first year of their existence, the sputum and gastric contents were consistently negative for tubercle bacilli, and the clinical impression was that these lesions represented spread of sarcoma. This impression was changed when one of the nodules cavitated and *M. tuberculosis* was cultured from the sputum. In retrospect, the lesions also show a resemblance to some cases of pulmonary torulosis (5); spontaneous resolution of pulmonary cryptococcosis has been reported.

The repeated courses of X-ray therapy and the nitrogen mustard administration may have played a part in further reducing resistance to infection in this patient; these various effects cannot be clearly defined. Adrenal cortical hormones were also administered but, since they were instituted after the development of frank meningitis, no predisposing role can be ascribed to them. The judiciousness of prednisone therapy in a patient such as this might well be questioned; it has been noted that cortisone in large doses has an adverse effect on experimental cryptococcosis (6). However, it was believed that the patient's situation was quite extreme and that death was imminent at the time steroids

were begun. The addition of prednisone seemed to reduce toxicity and coincided with considerable remission of symptoms.

The role of sulfonamides in producing the first remission is difficult to assess. Despite the continued isolation of *C. neoformans*, there was objective improvement in the cerebrospinal fluid which may have been due to the sulfonamides. However, spontaneous remissions are sometimes seen in torula meningitis. Also, it is possible that the apparent improvement was due to prednisone, with reduced inflammation of the meninges.

Following relapse of the meningitis, disappearance of Cryptococci from the cerebrospinal fluid with amphotericin B therapy seems too striking to be merely coincidental. Regardless of any effect of therapy on the meningitis, the extremely reduced renal function and persistent bacteremia with drug resistant *E. coli* were believed to be the major factors in her death, and it is unreasonable to consider her death a failure in amphotericin B therapy. Promising results with amphotericin B have been reported in cryptococcal meningitis by others (7, 8).

Summary

A case of reticulum cell sarcoma complicated by meningitis has been presented, and is of interest in that *Cryptococcus neoformans* and *M. tuberculosis* were isolated from the cerebrospinal fluid simultaneously. This is the second reported instance of such combined meningeal infection. It seems likely that the primary cause of meningitis was the Cryptococcus. Involvement of the subarachnoid space by *M. tuberculosis* occurred while the patient was receiving single-drug therapy with isoniazid for pulmonary tuberculosis and while the pulmonary disease was under good control.

Sumario

Meningitis Criptocócica y Tuberculosa Combinadas como Complicación del Sarcoma Reticulocelular: Presentación de un Caso

Preséntase un caso de sarcoma reticulocelular complicado por meningitis, siendo interesante el hecho de haberse aislado simultáneamente del líquido cefalorraquídeo el *Cryptococcus neoformans* y el *M. tuberculosis*. Este el segundo caso descrito de infección meníngea combinada de tal género. Parece probable que la causa primaria de la meningitis fuera el criptococo. La invasión del espacio subaracnoideo por el *M. tuberculosis* ocurrió mientras el enfermo recibía tratamiento con una droga, isoniacida, por tuberculosis pulmonar y mientras la afección pulmonar estaba bien dominada.

Resume

Méningite tuberculeuse et cryptococcique mixte compliquant un sarcome du réticulum: Observation d'un cas.

Présentation d'un cas de sarcome du réticulum compliqué de méningite. L'isolement simultané de *Cryptococcus neoformans* et de *M. tuberculosis* dans le liquide céphalo-rachidien confère à ce cas un intérêt particulier. C'est la seconde fois qu'une infection méningée mixte a été rapportée. Il paraît probable que le *Cryptococcus* était la cause initiale de la méningite. L'atteinte de l'espace subarachnoïdien par *M. tuberculosis* s'est produite alors que le malade était exclusivement traité par l'isoniazide pour une tuberculose du poumon et que l'affection pulmonaire était bien contrôlée.

Acknowledgment

The writers are indebted to R. S. Benham for bacteriologic observations and for the photomicrograph in figure 4. They also wish to acknowledge the assistance of Dr. W. H. Kirsten, who performed the autopsy in this case.

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TRANSITION FROM OPEN PULMONARY TUBERCULOSIS TO SARCOIDOSIS[1]

A. W. LEES

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Introduction

The development of tuberculosis in patients suffering from sarcoidosis has frequently been noted; but the transition from open pulmonary tuberculosis to sarcoidosis has so rarely been recorded that the following example seems worth reporting.

Case Report

A 22-year-old male was admitted to the hospital on May 8, 1956. He had had a pain in the right side, slight dyspnea for ten days, and a productive cough for two to three weeks. His temperature was 101°F.

Physical examination suggested the presence of a small right pleural effusion. This was confirmed on May 9, 1956, by chest roentgenography (figure 1) which also showed consolidation in the right lower lobe and bilateral perihilar infiltration. Acid-fast bacilli were seen on direct examination of the sputum on May 9 and 10, and tubercle bacilli susceptible to streptomycin, isoniazid, and PAS were cultured from specimens of pleural fluid and bronchial washings obtained on May 11 and 12, respectively.

Treatment with *o*-hydroxybenzal isonicotinyl hydrazone (Nupasal®), 2 gm. daily, and with PAS, 20 gm. daily, was commenced on May 15, 1956. A roentgenogram obtained on August 16, 1956, showed that the right pleural effusion had absorbed and that there had been some resolution of the disease in the right lower lobe. The general condition of the patient steadily improved and by September 10, 1956, was excellent. His erythrocyte sedimentation rate, which was 26 mm. (Westergren) in one hour on admission, had returned to normal by September, and his weight had gone from 114 to 154 pounds over the same period. A chest film on October 10 of the same year, however, showed that infiltration had developed throughout both lung fields and, although the patient remained perfectly well, a subsequent film on November 14 (figure 2) showed still further deterioration. Repeated cultures of bronchial washings and laryngeal swabs were negative for tubercle bacilli. Bronchoscopy was carried out on November 27, but showed no abnormality. The following was contained in a report on a biopsy specimen of bronchial mucosa sent for histologic examination:

> The material submitted consists of a portion of bronchial mucosa. The stroma shows severe edema, the appearance of which is similar to and suggestive of the mucoid degeneration often found in the stroma of smooth mucous polyps.

No yeasts or fungi were cultured from bronchial washings, and no eosinophilia or other blood abnormality was found. The bones of the hands and feet were roentgenographically normal, and the plasma proteins were within normal limits. A Mantoux test was negative with 1:1,000 dilution of OT (November 28, 1956) and with 1:100 dilution of OT (December 6). On December 20 a small portion of the superior segment of the lingula was removed through a 4-inch incision in the fifth left interspace (T. Welsh). At thoracotomy accessible lung tissue felt nodular. The report on histologic examination of the lingular biopsy specimen was:

> The pattern of normal lung alveoli is almost completely destroyed and is replaced by fibro-cellular tissue which contains many giant and endothelioid cells. A considerable amount of fibrous tissue is present. No caseation is seen, and I consider

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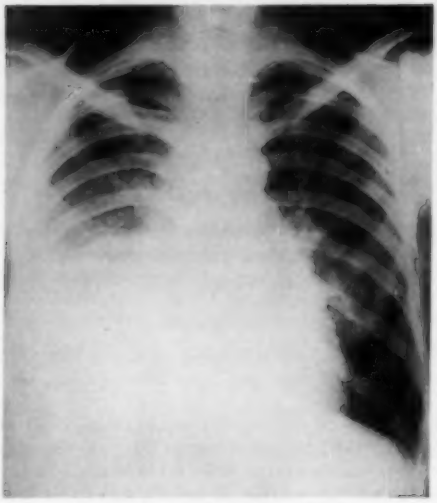

FIG. 1. May 9, 1956. Right pleural effusion, consolidation of right lower lobe, perihilar infiltration.

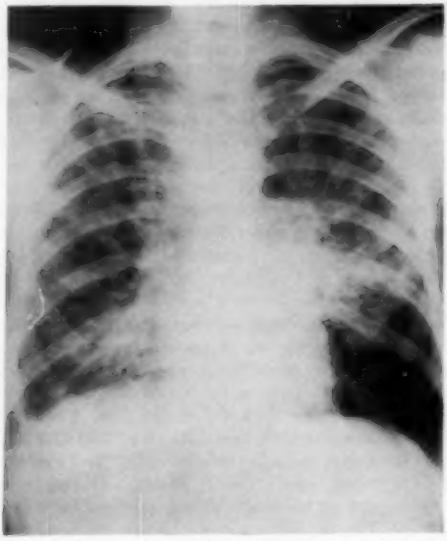

FIG. 2. November 14, 1956. Widespread infiltration in both lungs.

the histological features of the lesion to be those of sarcoidosis. No tubercle bacilli or other organisms have been seen in specially stained sections.

Culture of lingular biopsy material for *M. tuberculosis* was negative.

Subsequent progress: On December 20, the patient's treatment was changed to daily administration of 1 gm. of streptomycin, 400 mg. of isoniazid, and 15 gm. of PAS. His general condition continued to be excellent, the appearance of the chest roentgenogram did not alter, and the erythrocyte sedimentation rate was never elevated. The plasma proteins remained within normal limits, and the weight remained steady. Repeated cultures of bronchial washings and laryngeal swabs were negative for *M. tuberculosis*, and the Mantoux test remained negative to 1:100 OT. There was no dyspnea. On February 26, 1957, the patient's vital capacity was 4,100 cc. and the 0.75-second forced expiratory volume was 73 per cent of this (normal, 70 per cent). The patient's maximal breathing capacity was 89 per cent of predicted. He was discharged from the hospital on February 27.

The patient has been seen regularly since then and, apart from some slight resolution of the widespread fibrosis in the lung fields, there has been no change in his condition. Bronchial washings and laryngeal swabs have been regularly cultured for *M. tuberculosis*, with negative results. A Mantoux test with 1:100 OT on February 13, 1958, was negative. The vital capacity on that date was 4,300 cc. and the 0.75-second forced expiratory volume, 67 per cent of this.

Comment

The presenting features of the patient's illness, i.e., loss of weight, productive cough, pyrexia, raised erythrocyte sedimentation rate, the presence of tubercle bacilli in pleural fluid, and respiratory secretions, were typical of pulmonary tuberculosis. The response to treatment also seemed typical until chest films showed the development of widespread disease in both lungs despite satisfactory improvement of the general condition. The persistent absence of tubercle bacilli in respiratory secretions and the negative tuberculin reaction suggested a diagnosis of sarcoidosis, and this was confirmed by lung biopsy.

Instances of bacteriologically proved pulmonary tuberculosis preceding sarcoidosis are rare; single cases have been reported by Hoyle (1), Lindig (2), and Emerson and Young (3). Refvem (4) reported an example of sarcoidosis preceded by a pleural effusion with tubercle bacilli in the fluid. In these four reported cases the tuberculin test was positive initially and remained positive after the development of sarcoidosis. In the present case the tuberculin reaction during the phase of open tuberculosis was not tested, but after sarcoidosis had developed it was negative and has remained negative for eighteen months.

Summary

Tubercle bacilli were cultured from the respiratory secretions and pleural fluid of a 22-year-old male with pulmonary tuberculosis and a right pleural effusion. After five months, when chemotherapy had caused reversal of infectiousness and restored the general condition to normal, widespread infiltration appeared in the lung fields. Lung biopsy proved that this was due to the development of sarcoidosis. The initial tuberculin reaction was not known; when sarcoidosis developed it was negative, and it has remained negative for eighteen months, to the present time.

Sumario

Transición de la Tuberculosis Pulmonar Abierta a la Sarcoidosis

Se cultivaron bacilos tuberculosos de las secreciones respiratorias y del líquido pleural de un varón de 22 años, que tenía tuberculosis pulmonar y un derrame en el lado derecho

de la pleura. A los cinco meses, cuando la quimioterapia había hecho virar la infecciosidad y volver a lo normal el estado general, apareció una infiltración generalizada en los campos pulmonares. La biopsia pulmonar demostró que esto se debía a la aparición de sarcoidosis. No se sabe cuál fué la reacción inicial a la tuberculina; cuando apareció la sarcoidosis era negativa y así ha permanecido por dieciocho meses hasta la fecha actual.

Resume

Passage d'une tuberculose pulmonaire ouverte à la sarcoïdose

Des bacilles tuberculeux ont été cultivés à partir de sécrétions des voies respiratoires et de liquide pleural dans un cas de tuberculose pulmonaire avec épanchement pleural droit chez un homme de 22 ans. Au bout de cinq mois, alors que la chimiothérapie avait entraîné la "négativation" de l'expectoration et ramené l'état général à la normale, une infiltration largement étendue est apparue dans les champs pulmonaires. La biopsie pulmonaire a démontré que ce phénomène était dû au développement d'une sarcoïdose. La réaction initiale à la tuberculine n'était pas connue; mais au moment où la sarcoïdose s'est développée cette réaction était négative et, depuis dix-huit mois, elle est demeurée négative.

REFERENCES

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(3) Emerson, P. A., and Young, F. H.: Sarcoidosis following tuberculosis, Tubercle, 1956, *37*, 116.

(4) Refvem, O.: The pathogenesis of Boeck's disease (sarcoidosis): Investigations on the significance of foreign bodies, phospholipids, and hypersensitivity in the formation of sarcoid tissue, Acta med. scandinav., 1954, Supplement No. 294.

Notes

AN EVALUATION OF CHEMOTHERAPY IN PULMONARY TUBERCULOSIS

Part I: High Doses of Isoniazid with PAS and Pyridoxine

This report concerns the clinical results in patients with active pulmonary tuberculosis who have been given high doses of isoniazid and para-aminosalicylic acid (PAS). The observations were made with respect to disappearance of tubercle bacilli from the sputum, cavity closure, and roentgenographic improvement. In addition, observations of drug toxicity and the concentrations of free isoniazid attained in the blood are presented.

One hundred and seventeen consecutively admitted patients whose cases met the following criteria were selected: active pulmonary tuberculosis; no previous chemotherapy; or less than two months of chemotherapy prior to admission. Thirty-three of the 117 patients were excluded from the study. Seventeen patients were started on other drug regimens because of coexistent renal tuberculosis or because the admitting physician inadvertently chose another drug regimen. Four patients were excluded because of toxic reactions to isoniazid as follows: dizziness, fever, skin rash, and toxic hepatitis with jaundice. Ten patients were dropped from the study following reactions to PAS which occurred within the first month of treatment. Toxic reactions to PAS included fever, skin rash, gastrointestinal intolerance, and one case of exfoliative dermatitis.

After these 33 patients (including those with toxic drug reactions) were excluded, 84 patients remained for analysis in this study.

Thirty-five of the 84 patients remaining in the study had received no treatment prior to admission. Forty-nine received less than two months of chemotherapy before admission, with an average duration of 1.03 months of preadmission chemotherapy.

By arbitrary decision, patients with noncavitary disease were given 10 mg. of isoniazid per kg. per day, and patients with cavitary disease were given 16 mg. of isoniazid per kg. per day in three divided doses. Thirty-seven received 10 mg. of isoniazid, 40 received 16 mg. of isoniazid, and 7 patients with initial inadequate serum concentrations of free isoniazid received 18 mg. of isoniazid per kg. per day.

All patients received 100 mg. of pyridoxine and 12 gm. of PAS daily. The PAS was administered in the form of 5.57 gm. of sodium PAS in freshly manufactured packets given three times daily. Para-aminosalicylic acid is a relatively unstable compound and tends to produce degradation products under the influence of light, heat, and moisture. In order to prevent the possible association between altered products of PAS and gastrointestinal intolerance, all patients were given this drug within two months of its manufacture. The sodium salt of PAS was sealed under nitrogen in polyethelene packets and stored in a dark, cool area until used.

In 64 of the 84 cases, the concentration of free isoniazid in the blood (serum) was determined six hours after one-third of the total daily dose of isoniazid and PAS was administered. The values for free isoniazid in 16 cases (25 per cent) were between 0.3 and 0.8 γ per ml. of serum, and in 48 (75 per cent), values between 1.0 and 6.5 γ of free isoniazid per ml. of serum were obtained. The free isoniazid was determined by use of a chemical and biologic method of assay. The bioassay method is a serial tube dilution technique as described by Morse and associates[1] and as used at the Research and Development Service

[1] Morse, W. C., *et al.*: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 283.

TABLE 1

BACTERIOLOGIC STUDIES

Eighty-Four Cases

Number of Months of Treatment	Number of Cases Studied	Number of Cases with Cultures Negative for Tubercle Bacilli	Per Cent of Cases with Cultures Negative for Tubercle Bacilli
0 (on admission)	84	20	23.8
2	84	70	83.3
3	84	76	90.5
4	84	80	95.2
5	79	77	97.5
6	64	63	98.5
8	32	32	100

All cases lost after four months were negative before four months.

TABLE 1A

DISAPPEARANCE OF TUBERCLE BACILLI FROM SPUTUM

(64 Patients with Sputum Positive for *M. tuberculosis* on Admission)

Number of Months of Treatment	All 64 Cases	Minimal Cases	Moderately Advanced Cases	Far Advanced Cases
	per cent	*per cent*	*per cent*	*per cent*
2	78	90	83	53.8
6	97.7	100	96.6	100

of Fitzsimons Army Hospital.[2] The chemical method of determining free isoniazid in serum is the procedure described by Maher and associates[3] and modified by Leifheit.[4] The chemical procedure utilized in this study was critically analyzed and compared with duplicate bioassay tests in a separate study. The chemical method as practiced in this study was found to be just as reliable as the biologic assay technique.[5] The chemical method was used in 51 cases and the biologic method was used in 13 cases.

All 84 patients were observed for four months, 79 were observed for five months, 63 for six months, and 32 for eight months.

Observations with respect to drug reactions were recorded in a larger group of cases. In 329 patients receiving 10 to 16 mg. of isoniazid per kg. per day, the incidence of toxic reactions to isoniazid was 1.8 per cent. All 329 patients were given 100 mg. of pyridoxine daily, and the periods of observation varied between two and nine months. No cases of peripheral neuropathy were observed. In 303 patients who received the equivalent of 12 gm. of PAS daily, 8.9 per cent developed toxicity. This 8.9 per cent of patients with toxicity to PAS includes those who developed gastrointestinal intolerance which precluded further administration of the drug. The incidence of gastrointestinal intolerance to PAS was 4.9 per cent.

The incidence of cultures positive for *M. tuberculosis* at the time of admission was: 50

[2] Laboratory Methods for Tuberculosis Bacteriology, Report No. 1, 1957, Research and Development Service, Fitzsimons Army Hospital, Denver, Colorado.

[3] MAHER, J. R., *et al.*: Am. Rev. Tubere., 1957, *76*, 852.

[4] LEIFHEIT, H. C.: Unpublished data.

[5] BERTE, S. J., *et al.*: Unpublished data.

per cent of 20 minimal cases, 80.5 per cent of 51 moderately advanced cases, and 100 per cent of 13 far advanced cases. The patients with negative cultures on admission were established as cases of active pulmonary tuberculosis on the basis of the clinical and roentgenographic findings.

In table 1, the percentage of all patients with sputum cultures negative for tubercle bacilli is tabulated. Reversal of infectiousness occurred early in the course of treatment. More than 90 per cent of patients remained in noninfectious status after the third month of therapy, and 98.5 per cent had no tubercle bacilli in the sputum at six months. The patients with cultures negative for tubercle bacilli are listed in table 2 with regard to extent of disease. In all cases of minimal tuberculosis the sputum remained negative for *M. tuberculosis* after two months of therapy. The sputum was likewise rendered noninfectious in more than 90 per cent of the moderately advanced cases after two months, and in 90 to 100 per cent of the far advanced cases after five and six months of observation. All patients lost from the study after four months had sputum cultures negative for tubercle bacilli prior to the four-month observation point. Consequently, the total percentage of patients rendered noninfectious by the sixth month of therapy would not have been affected had these "lost cases" been included.

All cavities that appeared to be closed on postero-anterior and apical roentgenograms were studied with planigrams. In 3 cases, the cavities appeared to have closed by examination of conventional films, but appeared to be open on planigraphic study. These were recorded as open cavities in this report.

TABLE 2

BACTERIOLOGIC STUDIES

Minimal, Moderate, and Far Advanced Cases

Number of Months of Treatment	Minimal		Moderately Advanced		Far Advanced	
	Number of Cases Studied	Per Cent Negative for Tubercle Bacilli	Number of Cases Studied	Per Cent Negative for Tubercle Bacilli	Number of Cases Studied	Per Cent Negative for Tubercle Bacilli
0 (on admission)	20	50	51	19.5	13	0
2	20	95	51	86.3	13	53.8
3	20	100	51	90.2	13	76.9
4	20	100	51	96.1	13	84.6
5	18	100	50	98	11	90.9
6	15	100	39	97.4	9	100

TABLE 3

CAVITY CLOSURE

(Forty-Seven Cases)

Number of Months of Treatment	Number of Cases Studied	Number of Cavities Closed	Per Cent of Cavities Closed
2	47	4	8.5
3	47	10	21.3
4	47	17	36.2
5	44	20	45.5
6	36	21	58.4

No cavities closed between six to eight month period.

TABLE 4

CAVITY CLOSURE BY SIZE

Number of Months of Treatment	Per Cent Closed		
	0.9–2.0 Cm. (29 Cases)	2.5–3.5 Cm. (13 Cases)	4.0–8.0 Cm. (5 Cases)
3	31.1	0	20
4	44.8	23.1	20
5	48.2	38.4	20
6	48.2	46.2	20

Forty-seven of the 84 patients had cavities and, as noted in table 3, in 58.4 per cent (21 cases) the cavities were closed within six months of the start of therapy. It was interesting to observe that a very early response toward closure occurred in the 21 of the 47 cavities that did close. Eighty-one per cent closed in four months, and 95.2 per cent were closed by the fifth month. Cavity closure by size is illustrated in table 4. It was noted that most cavities less than 2 cm. in size that did close did so by the fourth month. The maximal closure rate in cavities between 2.5 and 3.5 cm. occurred at six months. There were only 5 cavities more than 4 cm. in size and only one of these closed. Four of the 21 cavitary cases that closed did so between the fourth and sixth months. Eleven patients with cavitary disease were lost from the study after four months. Of these 11 lost, 5 represented open cavities at the time they were lost. Had all 11 patients been followed for six months, and had the 5 patients with open cavities not closed their cavities, the cavity closure rate could not have been less than 45 per cent. Most studies of this nature do not require planigraphic evidence of cavity closure. If planigrams were not used as criteria for cavity closure, 24 instead of 21 cavities would have been recorded as closed. Under the latter circumstances, the cavity closure rate would not have been below 51 per cent at six months even with the inclusion of those open cavities which were lost from study before six months.

Cases of moderate to marked roentgenographic improvement are listed in table 5. Moderate to marked roentgenographic improvement was graded as improvement greater than 50 per cent from the time of admission, and with no areas of worsening. Cavities were rated separately. Approximately 25 per cent of the patients had moderate to marked roentgenographic improvement after three months of treatment. In 80 to 100 per cent of the moderate and far advanced cases, and in more than 75 per cent of all cases, moderate to marked roentgenographic improvement had occurred at the five- to six-month observation point. The lower per cent (61.2) of minimal cases in which improvement was evident by six months is attributed to the natural tendency to be more critical when it has to be decided that greater than 50 per cent of a minimal lesion has resolved.

As this report is a pilot study, no randomized control group of patients on low doses of isoniazid plus PAS is available. For purposes of general comparison, other published data were reviewed. More than 700 patients reported in the Veterans Administration-Armed Forces cooperative study[6] were treated with 300 mg. of isoniazid and 12 gm. of PAS daily, and analyzed in a similar fashion to the 84 cases described herein. Although there are eight and one-half times as many cases in the Veterans Administration-Armed Forces study as in this report, the distribution of cases is similar. Both studies encompassed an equal

[6] LIVINGS, D. G.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 20.

TABLE 5

PATIENTS WITH MODERATE TO MARKED ROENTGENOGRAPHIC IMPROVEMENT

	At 3 Months		At 5-6 Months	
	Number of Cases	Per Cent Improved	Number of Cases	Per Cent Improved
Minimal	20	30	18	61.2
Moderately advanced	51	21.6	50	80
Far advanced	13	23.1	11	100
All cases	84	23.8	79	78.5

per cent of cavitary cases, and moderately advanced cases were predominant in each group. The per cent of cases with sputum cultures negative for tubercle bacilli at six months in the Veterans Administration-Armed Forces cooperative study was as follows: all cases, 79.8 per cent; minimal cases, 84 per cent; moderately advanced cases, 86 per cent; and far advanced cases, 71 per cent. When these data are compared with the results of the present study (*see* tables 1 and 2), it may be seen that tubercle bacilli disappeared from the sputum with high doses of isoniazid in at least 15 to 20 per cent more cases. Moderate to marked roentgenographic improvement in minimal, moderate, and far advanced cases increased 20 to 30 per cent after six months of treatment in the 84 patients treated with large doses of isoniazid reported herein. Cavity closure at six months was apparently enhanced from 8 to 16 per cent in cavities less than 4.0 cm. in size by higher doses of isoniazid. There are certainly valid objections to a comparison of one series containing more than 700 cases with another containing 84 cases. Nevertheless, in a pilot study such as is reported herein, it is necessary to evaluate the results of treatment in terms of previously established experience. On this basis, there appears to be a decided general tendency toward an enhanced treatment result when high doses of isoniazid and PAS are employed.

In a recent study by McCune and associates,[7] the use of high doses of pyridoxine and isoniazid was studied in mice. There appeared to be a pyridoxine antagonism of the microbiologic effect of isoniazid after four weeks. In the study of McCune and associates, the dose of pyridoxine given was three times greater than the dose of isoniazid on a weight-for-weight basis. In the 84 cases reported herein, the dose of isoniazid varied between 600 and 1,500 mg. daily. As 100 mg. of pyridoxine were administered, the dose of isoniazid for each patient was six to fifteen times greater than the dose of pyridoxine. Although pyridoxine undoubtedly antagonizes the antimicrobial effect of isoniazid, the mechanism is not completely understood. Data in the present report are self-evident, and strongly suggest that high doses of isoniazid, PAS, and pyridoxine can provide clinical results which are better in all phases of evaluation than results observed previously with low doses of isoniazid and PAS without pyridoxine. Dosages given to animals cannot be applied directly to humans. However, the ratio of isoniazid to pyridoxine in this report was significantly higher than that used in the study by McCune and co-workers. It is believed that any antagonism produced by pyridoxine was masked by the high dosage of isoniazid in relation to pyridoxine.

Mitchell and Bell[8] indicated that an adequate serum-free isoniazid concentration (six hours after one-third of the total daily dose of isoniazid and PAS) is probably between 0.4 and 0.8 γ per ml. The latter writers recommend that the dose of isoniazid be adjusted to

[7] MCCUNE, R., *et al.*: Am. Rev. Tuberc., 1957, *76*, 1,100.

[8] MITCHELL, R. S., AND BELL, J. C.: New England J. Med., 1957, *257*, 1,066.

provide a concentration of 0.8 γ of free isoniazid per ml. at six hours after ingestion. Twenty-two (46 per cent) of Mitchell and Bell's patients were considered relatively rapid inactivators of isoniazid because serum concentrations of free isoniazid were less than 0.8 γ per ml. In the present study, only 25 per cent of 64 patients tested had serum concentrations between 0.3 and 0.8. It should be realized that all patients received 12 mg. of isoniazid per kg. per day in Mitchell and Bell's study. In the present report, 42 per cent of patients tested received 16 or more mg. of isoniazid per kg. daily. The higher doses of isoniazid in the present study account for the greater per cent of patients with adequate concentrations of free isoniazid.

In summary: A general comparison with previously published results using a low dosage of isoniazid with PAS suggests that the use of high doses of isoniazid with PAS provides superior results in all phases of clinical evaluation.

Seventy-five per cent of the patients had adequate free-serum isoniazid concentrations. One-fourth of the patients had detectable concentrations between 0.3 and 0.8 γ of free isoniazid per ml. six hours after one-third of the total daily dose of isoniazid and PAS was given. These patients were considered as rapid acetylators. No correlation could be made between free-serum isoniazid concentrations and reversal of infectiousness because more than 97 per cent of all patients had sputum negative for tubercle bacilli on culture at six months.

The occurrence of peripheral neuropathy is not to be feared when 100 mg. of pyridoxine are administered once daily. Perhaps a lower dose of pyridoxine might be just as effective. Antagonism of biologically active isoniazid by pyridoxine was not detectable in the clinical results. The incidence of gastrointestinal intolerance due to PAS was less than 5 per cent. This is attributed directly to the use of a freshly manufactured preparation delivered to patients within two months of its manufacture.

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AN EVALUATION OF CHEMOTHERAPY IN PULMONARY TUBERCULOSIS

Part II: Daily Streptomycin Plus High Doses of Isoniazid with PAS and Pyridoxine

The method of selection of cases and the criteria for determining cavity closure and roentgenographic improvement in the present study of the chemotherapy of tuberculosis are the same as those described in the previous paper of this series.[1] The purpose of the present study is to investigate the value of adding daily streptomycin to regimens consisting of high doses of isoniazid and para-aminosalicylic acid (PAS).

Seventy-seven consecutively admitted patients with active pulmonary tuberculosis, with no previous chemotherapy or less than two months of previous chemotherapy, were selected. Seven patients were excluded: 2 were lost from the study within two months; 4 developed toxicity to PAS; and streptomycin was discontinued in one because of furuncles at the site of injection. Two other patients developed toxicity to streptomycin, but the drug was not discontinued. One of these developed a skin rash and fever, and was successfully desensitized to streptomycin. The other developed dizziness and flushing which were relieved by pyribenzamine. All patients were evaluated with an audiogram and caloric stimulation tests before and after the course of streptomycin.

Of the 70 patients remaining in the study, 27 had had no previous drug therapy and 43 had had less than two months of preadmission chemotherapy. In the latter group, the average duration of drugs before admission was 0.92 months.

All 70 patients received the following drug therapy: streptomycin, 1 gm. daily for ninety days; isoniazid, 10 to 18 mg. per kg. per day; PAS, 12 gm. daily (given in the form of 5.57 gm. of sodium PAS three times daily in freshly manufactured packets); and 100 mg. of pyridoxine once daily. Twenty-two (31.4 per cent) of the patients received 10 mg. of isoniazid per kg. per day; 40 (57.2 per cent) received 16 mg. of isoniazid per kg. per day; and 8 (11.4 per cent) received 18 mg. per kg. of isoniazid daily.

The concentration of free isoniazid in serum was determined by the chemical and biologic assay method as described in the previous report.[1] Determinations were made in 63 of the 70 patients. Serum was obtained six hours after one-third of the total daily dose of isoniazid and PAS was administered. The chemical method was used in 37 cases, and the biologic method of assay was used in 26 cases. Streptomycin was discontinued for three days prior to determining the free isoniazid in the blood. In 44 patients (69.8 per cent), the concentrations of free isoniazid were between 1.0 and 6.2 γ per ml. In the remaining 19 patients, the isoniazid concentrations ranged between 0.0 and 0.8 γ per ml. of serum.

All 70 patients were followed for four months from the start of therapy, 67 were followed for five months, and 57 for six months.

In 28.6 per cent of the series, the patients' sputum cultures were negative for *M. tuberculosis* at the time of admission to the study. These cases were accepted as active tuberculosis by all other criteria. As seen in table 1, 81.5 per cent of the series of patients had noninfectious sputum after two months of treatment, and 98.3 per cent were noninfectious after six months of treatment. In table 2, it may be observed that the sputum cultures remained negative for tubercle bacilli in all of the minimal cases after two months of treatment. In more than 90 per cent of the patients with moderately advanced pulmonary tuberculosis the sputum cultures were consistently negative for tubercle bacilli after three months of chemotherapy, and in all cases the examinations were negative after six months of treatment. More than 91 per cent of a small group of patients with far advanced disease had noninfectious sputum after six months of therapy.

[1] Berte, S. J., *et al.*: Am. Rev. Tuberc., 1958, *78*, 773.

TABLE 1

BACTERIOLOGIC STUDIES

Seventy Patients

Number of Months of Treatment	Number of Cases Studied	Number of Cases of Cultures Negative for Tubercle Bacilli	Per Cent of Cases of Cultures Negative for Tubercle Bacilli
0 (on admission)	70	20	28.6
2	70	57	81.5
3	70	62	88.6
4	70	66	94.3
5	67	64	95.5
6	57	56	98.3

TABLE 2

BACTERIOLOGIC STUDIES

Minimal, Moderate, and Far Advanced Cases

Number of Months of Treatment	Minimal		Moderately Advanced		Far Advanced	
	Number of Cases Studied	Per Cent Negative for Tubercle Bacilli	Number of Cases Studied	Per Cent Negative for Tubercle Bacilli	Number of Cases Studied	Per Cent Negative for Tubercle Bacilli
0 (on admission)	12	66.6	44	27.3	14	0
2	12	100	44	84.1	14	57.2
3	12	100	44	93.2	14	64.3
4	12	100	44	97.7	14	78.5
5	11	100	42	97.6	14	85.7
6	10	100	35	100	12	91.7

TABLE 3

CAVITY CLOSURE

(Thirty-Two Cases)

Number of Months of Treatment	Number of Cases Studied	Number of Cavities Closed	Per Cent of Cavities Closed
3	32	8	25
4	32	17	53.1
5	32	19	58.3
6	27	17	63.0

Eighty-five per cent of cavities that closed did so within four months.

Two cavities that closed on conventional roentgenograms remained open on planigrams.

Thirteen patients were lost from the study between the fourth and sixth months of chemotherapy. In 12 of these patients, the sputum cultures had been negative for *M. tuberculosis* prior to the fourth month of study. Only one patient, "lost" after five months, was discharging tubercle bacilli in the sputum on the last examination. An open cavity was present in this case. If the 13 patients lost from observation had been followed for six months, their inclusion would not have significantly altered the percentage of patients with cultures negative for tubercle bacilli at the sixth-month observation point.

TABLE 4

CAVITY CLOSURE BY SIZE

Number of Months of Treatment	Per Cent Closed		
	0.8–2.0 Cm. (19 Cases)	2.5–3.5 Cm. (10 Cases)	4.0–7.0 Cm. (3 Cases)
3	26.3	20	33
4	58.0	50	33
5	63.2	60	33
6	68.3	60	66

TABLE 5

PATIENTS WITH MODERATE TO MARKED ROENTGENOGRAPHIC IMPROVEMENT

Type of Case	At 3 Months		At 5–6 Months	
	Number of Cases	Per Cent Improved	Number of Cases	Per Cent Improved
Minimal	12	33.3	11	54.5
Moderately advanced	44	38.6	42	90.5
Far advanced	14	14.3	14	93.0
All cases	70	32.8	67	85.0

The over-all rate of cavity closure is illustrated in table 3. Sixty-three per cent of cavities closed after six months, and the majority of these closed after only four months of treatment. It may be noted in table 4 that small and medium-sized cavities closed at about the same rate (60 to 68.3 per cent). Two of three cavities more than 4 cm. in size closed by the sixth month.

All of the 32 cavitary cases were studied for five months. Five cavitary cases were lost from the study between the fifth and sixth months. In only 2 of these cases was the cavity still open when the patient was lost from the study at the five-month period. Had all 5 cases been studied for six months, and had the latter cavities remained open at six months, the cavitary closure rate would have been 58.3 per cent. This is equal to the closure rate observed at five months in table 3.

The degree of moderate to marked roentgenographic improvement may be seen in table 5. Eighty-five per cent of all patients, and more than 90 per cent of those with moderate and far advanced lesions, demonstrated greater than 50 per cent roentgenographic improvement at the five- to six-month observation point.

* * *

In tables 6 through 10 the results from the previous study using high doses of isoniazid along with PAS are compared with the results of the triple-drug regimen used in the present study. As shown in table 6, there is no difference in the rate of reversal of infectiousness in either group. After six months of treatment, there exists a difference of less than 1 per cent in the number of patients whose sputum cultures were negative for tubercle bacilli. In table 7, bacteriologic studies are recorded in terms of extent of disease. There is less than a 3 per cent difference between the two-drug and the three-drug regimens when minimal and moderately advanced cases are compared in terms of sputum cultures after six months of treatment. The small number of far advanced cases does not justify an exact comparison. In table 8, the difference between the two regimens with respect to the

TABLE 6

BACTERIOLOGIC STUDIES

Two-Drug Regimen of Previous Study Compared with Three-Drug Regimen

Number of Months of Treatment	84 Cases High-Dosage Isoniazid and PAS Per Cent with Cultures Negative for Tubercle Bacilli	70 Cases Daily Streptomycin and High-Dosage Isoniazid and PAS Per Cent with Cultures Negative for Tubercle Bacilli
0	23.8	28.6
2	83.3	81.5
6	98.5	98.3

TABLE 7

BACTERIOLOGIC STUDIES

Two-Drug Regimen of Previous Study (84 Cases) Compared with Three-Drug Regimen (70 Cases)

Number of Months of Treatment	Minimal		Moderately Advanced		Far Advanced	
	High-Dosage Isoniazid and PAS	Daily Streptomycin, High-Dosage Isoniazid and PAS	High-Dosage Isoniazid and PAS	Daily Streptomycin, High-Dosage Isoniazid and PAS	High-Dosage Isoniazid and PAS	Daily Streptomycin, High-Dosage Isoniazid and PAS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
2	95	100	86.3	84.1	53.8	57.2
6	100	100	97.4	100	100	91.7
Number of cases in study	20	12	51	44	13	14

TABLE 8

CAVITY CLOSURE

Number of Months of Treatment	Two-Drug Regimen High-Dosage Isoniazid and PAS		Three-Drug Regimen Daily Streptomycin, High-Dosage Isoniazid and PAS	
	Number of Cases Studied	Per cent of Cavities Closed	Number of Cases Studied	Per Cent of Cavities Closed
3	47	21.3	32	25
6	36	58.4	27	63

TABLE 9

CAVITY CLOSURE BY SIZE AT SIX MONTHS

	Per Cent Closed		
	0.8–2.0 Cm.	2.5–3.5 Cm.	4.0–8.0 Cm.
Two-drug regimen (high-dosage isoniazid and PAS)	48.2 (29 cases)	46.2 (13 cases)	20 (5 cases)
Three-drug regimen (daily streptomycin, high-dosage isoniazid, and PAS)	68.3 (19 cases)	60 (10 cases)	66 (3 cases)

TABLE 10

PATIENTS WITH MODERATE TO MARKED ROENTGENOGRAPHIC IMPROVEMENT

	Per Cent Improved at Six Months			
	Minimal	Moderately Advanced	Far Advanced	All Cases
Two-drug regimen (high-dosage isoniazid and PAS)	61.2 (18 cases)	80 (50 cases)	100 (11 cases)	78.5 (79 cases)
Three-drug regimen (daily streptomycin, high-dosage isoniazid, and PAS)	54.5 (11 cases)	90.5 (42 cases)	93 (14 cases)	85 (67 cases)

rate of closure of all cavities is less than 5 per cent at six months. When a comparison of the two regimens is made in terms of cavity closure by size (table 9), more than 20 per cent more cavities less than 2 cm. in size closed in six months on the triple-drug regimen. A comparison of the two regimens by moderate to marked roentgenographic improvement is presented in table 10. More than three-quarters of all patients had moderate to marked improvement at six months, and there was no significant difference between those receiving the two-drug or the three-drug regimen.

In a recent report by Wier and associates,[2] 98 patients with moderately advanced or far advanced pulmonary tuberculosis were observed for more than six months and the results were analyzed in the same way as in the present study. In the Wier study, the streptomycin was not given daily, and lower doses of isoniazid and PAS were employed. A general comparison has been made between the study of Wier and associates and the present report. This was done in order to appraise any advantage that may be attributed to daily streptomycin and high doses of isoniazid in such a triple-drug regimen. There was no difference in the percentage of patients whose sputum cultures were negative for tubercle bacilli six months after the start of therapy. There appeared to be a significant difference with respect to cavity closures and roentgenographic improvements in the two studies. In this respect, a comparison with the Wier study indicates that in the present study 18 per cent more cavities closed after four months of treatment and 16.3 per cent more cavities closed after six months of treatment. Moderate to marked roentgenographic improvement was 15.6 per cent greater in the study reported herein.

In summary: In general, there appears to be no advantage to be gained by adding daily streptomycin for ninety days to drug regimens containing high doses of isoniazid and PAS. Nearly all comparative observations are close to a 5 per cent range difference. It was of interest, however, to note that 20.1 per cent more cavities less than 2 cm., and more cavities greater than 4 cm. in size, closed in six months when daily streptomycin was added to high doses of isoniazid and PAS.

Although reversal of infectiousness and roentgenographic improvement are not enhanced by daily streptomycin, it is stimulating to ponder whether or not daily streptomycin with high doses of isoniazid and PAS will produce better cavity closure than will high doses of isoniazid and PAS without streptomycin. A much larger study will be needed to answer this question.

A general comparison with other studies utilizing intermittent streptomycin and lower doses of isoniazid and PAS was made. There are indications that the substitution of daily

[2] WIER, J. A., *et al.*: Am. Rev. Tuberc., 1956, *73*, 117.

streptomycin and high doses of isoniazid in such a triple-drug regimen can enhance cavity closures and roentgenographic improvements.

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THE VITAL STAINING METHOD FOR THE RAPID ESTIMATION OF THE BACTERIAL COUNT OF BCG VACCINE

A rapid method for the estimation of the number of viable cells in BCG vaccine has been in routine use in this laboratory for more than a year.[1] The method has been found extremely useful in the control of fluid BCG vaccine since the results, which are available in three days, yield essentially the same results as the conventional plate count which takes three to four weeks or longer. The present paper reports in detail some of the results obtained and is an attempt to appraise the value of this technique.

The method has been described in detail elsewhere.[1] Briefly, 15 mg. of BCG vaccine are inoculated into 4.5 ml. of a modified Sauton medium which is incubated for twenty-four hours at 37°C. Then 0.25 ml. of 1 per cent neotetrazolium chloride is added and incubated for a further forty-eight hours at the same temperature. The suspension is thoroughly shaken and a 1:50 dilution is prepared in Erlenmeyer flasks containing steel balls. The diluted material is shaken gently and 0.1 ml. is placed in 6 mm. stainless steel or glass cups which have been previously mounted on a glass slide with nail polish. After drying at 56°C. for several hours, the cup is removed and the slide fixed by flame. The metabolically active bacteria which have absorbed and reduced the neotetrazolium are stained dark purple and can be counted under an oil immersion objective using a calibrated ocular micrometer. The metabolically inactive bacteria which do not absorb the neotetrazolium remained unstained, and may be counterstained with saturated picric acid, acridin yellow, trypoflavine, or Bouin fixative.

In this study, three types of BCG vaccine were tested: vaccines prepared by the classic method and harvested on the fourteenth day of culture; vaccines prepared in the same manner but harvested on or before the tenth day; and freeze-dried vaccines harvested on the ninth day.[2] An example of the type of results obtained is shown in table 1, in which the results from five consecutive vaccines of each of the three types selected at random are given. With vaccines in group *A*, there is a close correlation between the colony count and the microscopic "cell-plus-clump" count. With vaccines in groups *B* and *C*, there is a close correlation between the colony count and the microscopic "individual cell" count. The organisms in group *C* did not form clumps.

During the course of this work, 83 vaccines were examined by this method. There was excellent agreement between the plate count and the microscopic cell-plus-clump count in 49 lots of 56 group *A* vaccines; in 7, there were variations of approximately 40 per cent or greater. There was excellent agreement between the colony and microscopic individual cell count in 17 of 20 group *B* vaccines, and similar good agreement was found in all of the 7 lots of freeze-dried (group *C*) vaccines. This is shown in table 2.

The method described and discussed in this report differs markedly from other vital staining techniques using tetrazolium salts[3, 4, 5] in that the organisms are counted directly. In the other techniques, the dye is extracted from the viable cells which have absorbed it, and estimations of the number of viable cells are made by colorimetrically determining the amount of dye absorbed. It is possible to estimate the approximate number of or-

[1] Eidus, L., *et al.*: Canad. J. Pub. Health, 1958, *1*, 18.
[2] Ungar, J., *et al.*: Brit. M. J., 1956, *11*, 568.
[3] Kanai, K., and Yanagisawa, K.: Jap. J. M. Sc. & Biol., 1954, *7*, 249.
[4] Vandiviere, H. M., *et al.*: Am. Rev. Tuberc., 1952, *66*, 95.
[5] Koch-Weser, D., *et al.*: Am. Rev. Tuberc., 1955, *71*, 556.

TABLE 1

EXAMPLE OF RESULTS OBTAINED WITH MICROSCOPIC AND PLATE-COUNTING TECHNIQUES

Vaccine Number	Age of Vaccine at Harvest	Plate Count* (per mg.)	Microscopic Count (per mg.)	
			Individual Cell	Cell Plus Clumps
A-1	14 days	3,833,000	24,650,000	4,635,000
A-2	14 days	1,800,000	6,595,000	2,095,000
A-3	14 days	1,800,000	8,300,000	2,650,000
A-4	14 days	1,125,000	22,000,000	2,420,000
A-5	14 days	1,793,000	8,788,833	2,833,000
B-1	10 days	26,250,000	31,275,000	4,775,000
B-2	10 days	28,300,000	36,000,000	6,750,000
B-3	10 days	23,150,000	29,250,000	5,500,000
B-4	10 days	30,500,000	24,580,000	4,672,875
B-5	10 days	30,500,000	24,259,000	8,360,275
C-1	Dried vaccine†	1,050,000	3,641,000	—
C-2	Dried vaccine†	2,120,000	2,560,000	—
C-3	Dried vaccine†	10,500	8,800	—
C-4	Dried vaccine†	24,000,000	30,840,000	—
C-5	Dried vaccine†	1,600,000	2,200,000	—

* Dubos oleic acid agar.

† Dried vaccine exhibited no clumps.

TABLE 2

COMPARISON OF RESULTS WITH PLATE COUNT AND MICROSCOPIC COUNT

Vaccine Group	Number Tested	Agreement Between Colony and Microscopic Count
A	56	49 (88%)
B	20	17 (85%)
C	7	7 (100%)
Total	83	73 (88%)

ganisms by use of a graph. This latter method, however, lacks the precision of the present technique.

The observation has been made[5, 6] that the staining ability of Mycobacteria varies with the different growth phases. While this may be true, it does not alter the efficacy of the present method since experience has shown that the microscopic count yields results comparable to those of the standard plate count.

A difference has been noted between vaccines harvested on the fourteenth day and those harvested on or before the tenth day. For vaccines in the former group, the standard plate count parallels the microscopic cell-plus-clump count, whereas with vaccines in the latter group it parallels the microscopic individual cell count. The explanation for this is not entirely clear, but it could be due to a number of causes, among them: The greater adhesiveness of older harvested vaccines might be such that the clumps do not break up

[6] KOCH-WESER, D., and EBERT, R. H.: J. Lab. & Clin. Med., 1955, *46*, 608.

as easily and form separate colonies as readily as do the earlier harvested vaccines; or the older harvested vaccines may have lost considerable vitality.

In summary: a vital staining method has proved very useful for determining the number of viable organisms in BCG vaccine. This method is rapid (two to three days), and close agreement was found in approximately 90 per cent of 83 vaccines examined. The differences noted in the remaining 10 per cent were not greater than those which have been observed at times between replicate plate tests carried out in this laboratory.

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A SEMI-SYNTHETIC AUTOCLAVABLE MEDIUM FOR A ROUTINE TUBERCULOSIS LABORATORY

It has been shown in special growth studies in different laboratories[1-4] that the requirements for the culturing of certain laboratory strains of tubercle bacilli vary for the different strains. Essential growth requirements of primary cultures of tubercle bacilli from specimens of patients are even more complex and difficult to define than are those of special laboratory strains. Growth-stimulating requirements vary with different degrees of drug resistance and enzyme activity, size of inoculum of viable organisms, and method of transporting and processing of specimens. Different strains of drug-susceptible tubercle bacilli in sputum also have varied requirements for primary growth.

The media usually employed for the routine primary isolation of tubercle bacilli from specimens present several serious deficiencies because their effectiveness depends upon enrichments which are variable in composition from time to time and are difficult to sterilize. Thus, egg yolk media, in general, vary in efficiency with the age of the eggs, the diet, and the breed of hens. In addition, coagulated egg media deteriorate on storage and become unsuitable for cultivation of certain strains of tubercle bacilli. Egg media are quite often contaminated during preparation and are not always sterilized by inspissation.

Serum and whole blood also vary in composition at different times and some batches of serum or whole blood-enriched media fail to detect certain strains of tubercle bacilli from concentrates containing viable organisms. The newer charcoal agars,[5, 6] although stable in composition, were found to be less effective for primary isolations of tubercle bacilli than either egg or serum media.[7]

Experiments were conducted in this laboratory to discover, if possible, a combination of easily acquired stable substances which would substitute for fresh egg yolk, which was thus far the most desirable enrichment in media for primary cultivation of tubercle bacilli.

One liter of base medium used in these studies consisted of the following substances:

$Na_2HPO_4.7H_2O$	2.9 gm.
KH_2PO_4	2.0 gm.
$Mg_2SO_4.7H_2O$	0.1 gm.
Iron and ammonium citrates	0.1 gm.
Citric acid (U.S.P.)	0.1 gm.
Dextrose (anhydrous)	4.0 gm.
Monosodium glutamate ($C_5H_8NNaOH_4$)	4.0 gm.
Glycerin (U.S.P.)	10 ml.
Potato starch	15 gm.
Agar	11 gm.
Water (distilled)	1 liter

To this base agar cholesterol, yeast extract, and whole powdered egg yolk were added separately and in various combinations and amounts. None of these substances produced satisfactory growths of newly isolated tubercle bacilli which grew luxuriantly on fresh egg

[1] Cohn, M. L., *et al.*: Am. Rev. Tuberc., 1954, *70*, 465.
[2] Baudisch, O., and Dubos, R. J.: Biochem. Ztschr., 1932, *245*, 278.
[3] Fisher, M. W.: Am. Rev. Tuberc., 1952, *66*, 626.
[4] Barry, V. C., *et al.*: Lancet, 1953, *1*, 979.
[5] Hirsch, J. G.: Am. Rev. Tuberc., 1954, *70*, 955.
[6] Whalen, J., and Mallman, W. L.: Am. Rev. Tuberc., 1954, *71*, 382.
[7] Peizer, L. R., *et al.*: Am. Rev. Tuberc., 1956, *74*, 428.

media. Growths on powdered egg yolk medium seemed somewhat encouraging, but it was difficult to sterilize and to keep the enrichment in even suspension.

Activated charcoals, Norrite A (Pfansteihl) and grade C 1-190-N, were tested in combination with cholesterol and yeast extract in the base agar, but the percentage of positive growths of the test strains of tubercle bacilli was poor compared to that on Löwenstein-Jensen and Peizer-Schechter media. When 10 per cent horse serum was added to samples of charcoal medium, the growths became satisfactory. Luxuriant growths of all test strains resulted upon addition of Triton WR 1339 to the serum charcoal agar.[8]

However, serum-enriched agar was not the solution sought. The serum had to be added to the sterilized medium under aseptic conditions, and the enriched agar carefully tubed under sterile precautions. The results of primary isolations of tubercle bacilli from concentrates were not consistent, often varying with different batches of serum.

Bovine albumin fraction V and sodium pyruvate were then added to the base agar, separately and in various combinations. Biotin was also introduced. Medium containing these three additives seemed, at times, to be suitable for the primary isolation of tubercle bacilli, but the results were not consistently satisfactory when compared to Löwenstein-Jensen and Peizer-Schechter media. In addition, bovine albumin was difficult to sterilize.

Starlac®, an instant-soluble milk product, was then tried as an enrichment along with sodium pyruvate and biotin. The resulting medium compared favorably with the egg media in its efficiency to isolate primary growths of tubercle bacilli from concentrates. It was found to be reproducible and fairly heat stable. One liter of the finally formulated medium is prepared as follows:

(*1*) Eleven grams of Difco agar are weighed and placed into 400 ml. of distilled water and the container is covered. The mixture is autoclaved to melt at 15 pounds for fifteen minutes.

(*2*) The following are weighed and added *in given order* to 450 ml. of distilled water in a sterile 2-liter Erlenmeyer flask:

Sodium glutamate ($C_5H_8NNaOH_4$)	40.0 gm.
Dextrose (anhydrous)[9]	4.0 gm.
$Na_2HPO_4.7H_2O$	2.9 gm.
KH_2PO_4	2.0 gm.
Yeast extract (Difco)[10]	0.2 gm.
Ferric ammonium citrate (U.S.P.)[11]	0.1 gm.
Citric acid (U.S.P.)	0.1 gm.
$MgSO_4.7H_2O$	0.1 gm.
Biotin 0.1 per cent sterile solution[12, 13]	4.0 ml.
Triton (WR 1339) 5 per cent sterile solution[13, 14]	3.0 ml.
Glycerine (U.S.P.)	10.0 ml.
Starlac (instant)[15]	7.0 gm.

The above ingredients are added to the water in the order listed, and brought to a slow boil.

(*3*) Fifteen grams of potato starch (U.S.P.) suspended in 82 ml. of distilled water are then added and the mixture is gently boiled for about three minutes, with stirring, until clear.

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[8] Hirsch, J. G.: Am. Rev. Tuberc., 1955, *71*, 894.

[9] Merck & Co., Rahway, New Jersey.

[10] Difco Laboratories, Detroit, Michigan.

[11] Mallinckrodt, New York, XIII Brown Pearls.

[12] Nutritional Biochemical Corp., Cleveland, Ohio.

[13] Sterilized by autoclaving at 15 pounds of pressure for fifteen minutes (121°C.) and stored in refrigerator.

[14] Winthrop-Stearns, Inc., New York, New York.

[15] Borden Co., New York, New York.

(4) Then the following are added:

Brilliant green: 0.1 per cent solution in H_2O: 1 ml.[16]

Pre-melted agar: 11 gm. in 400 ml. of distilled water (*see* above).

Sodium pyruvate (commercial grade)[17]: 5 gm. dissolved in 50 ml. of distilled water.

The agar is thoroughly mixed and divided into sterile test tubes in 10 ml. quantities by means of a pipetting machine with a sterilized tubing device. The pipetting is not done with sterile precautions, but care is taken to avoid gross contamination of the tubes. The tubes are loosely stoppered with pre-autoclaved corks. The tubed medium is then sterilized by placing the racks of medium tubes into the autoclave for five minutes at five pounds of pressure (110°C.). Excessive heat will deteriorate the medium. The tubes of medium are removed from the autoclave to a table as soon as the pressure is down to zero, are slanted, and left overnight at room temperature, and covered with brown paper to protect them from dust and direct sunlight. A sample number of medium tubes are incubated at 37°C. and another sample at room temperature, as a test of sterility. To avoid drying, the medium slants are securely corked before storage in the refrigerator.

Ninety-five per cent of the sputum and gastric specimens used in this study for inoculation of medium were transported and digested in 10 per cent hydrated ($12\ H_2O$) trisodium phosphate.[18] The remainder were processed by the NaOH-HCl digestion method. A cotton swab thoroughly dipped in sputum concentrate was used to inoculate each slant by vigorously streaking the agar surface about twelve times while rotating the swab at the same time.

The new medium was tested at various times on concentrated sputum specimens from isoniazid-treated patients in a comparison with the Löwenstein-Jensen and the Peizer-Schechter media. The results may be seen in tables 1 and 2.

A total of 22,844 specimens were cultured at four different intervals. After six weeks of incubation, 1,184 specimens were positive for tubercle bacilli on the egg media, and 1,175 on the semi-synthetic medium (table 1).

The results of the number of instances in which a positive culture was obtained on one medium which was negative on the other medium at the end of six weeks of incubation and after eight weeks of incubation may be seen in table 2.

At the end of six weeks of incubation, 65 cultures were positive for tubercle bacilli on the egg media and negative on the new medium. The converse occurred forty-nine times. At the end of eight weeks of incubation, 47 of 49 remained negative on the egg media, and 24 of 65 remained negative on the semi-synthetic medium.

There were 230 contaminated cultures on the egg media, 4 of which were positive for tubercle bacilli on the synthetic medium. There were 342 contaminated cultures on the synthetic medium, 5 of which were positive for tubercle bacilli on the egg media.

The comparisons as shown in tables 1 and 2 indicated that the difference in efficiency between the egg media and the new synthetic medium was small and probably not significant. The number of contaminated cultures, however, was about 1.5 times as great on the synthetic medium as on the egg media.[16]

Colony morphology with the synthetic medium is different at first from the reference type of dry rough growth on Löwenstein-Jensen, and is more like the morphology of young colonies of tubercle bacilli on Peizer-Schechter agar. The colonies are smooth and inclined to be mucoid during early growth. They become typically rough and dry when

[16] Since the completion of this study, by increasing the amount of brilliant green from 1 ml. of 0.1 per cent solution per liter to 0.25 ml. of a 1.0 per cent solution of dye, the number of contaminated cultures has been reduced on the synthetic medium to about 1.0 per cent.

[17] Matheson Coleman & Bell Division, East Rutherford, New Jersey.

[18] PEIZER, L. R., *et al.*: Am. Rev. Tuberc., 1954, *70*, 363.

TABLE 1

COMPARISON OF ISOLATIONS OF TUBERCLE BACILLI ON SEMI-SYNTHETIC MEDIUM AND ON EGG MEDIA

Number of Specimens Cultured	Egg Medium			Semi-synthetic Medium			Total Number Positive on Both Media	
		Incubation			Incubation		Incubation	
		6 Weeks	8 Weeks		6 Weeks	8 Weeks	6 Weeks	8 Weeks
1,288	Löwenstein-Jensen (L-J) Peizer-Schechter (P-S)	72	72	Semi-synthetic (S-S)	70	79	79	86
3,352	L-J and P-S	181	182	S-S	179	192	185	201
2,480	P-S	245	245	S-S	247	255	247	255
15,715	P-S	686	687	S-S	679	690	712	724
Totals 22,844*		1,184	1,186		1,175	1,216	1,223	1,266

* 230 specimens were contaminated on the egg medium, of which 4 were positive on the semi-synthetic medium.

372 specimens were contaminated on the semi-synthetic medium, of which 5 were positive on the egg medium.

See Footnote 9 for further information concerning contaminants.

TABLE 2

COMPARISON OF THE NUMBER OF CULTURES POSITIVE FOR M. TUBERCULOSIS ON EGG MEDIUM AND NEGATIVE ON THE SEMI-SYNTHETIC MEDIUM, AND VICE-VERSA

Incubation Time	Total Number of Cultures Positive on Egg Media	Total Number of Cultures Positive on the Semi-synthetic Medium	Number of Cultures Positive on Egg but Negative on Semi-synthetic Media	Number of Cultures Positive on Semi-synthetic but Negative on Egg Media
Weeks				
6	1,184	1,175	65	49
8	1,186	1,216	24	47

fully grown. Primary growth of about 75 positive specimens is clearly seen at a glance after three to four weeks of incubation. Cultures on this medium should be incubated for eight weeks before being reported as negative for tubercle bacilli (*see* table 2).

Triton WR 1339 (polyoxethylene ether) is a nontoxic wetting agent which enhances the growth of tubercle bacilli from small inocula in this medium[8]; it is partially the cause of the mucoid smooth colony morphology of young colonies of tubercle bacilli on the medium.

Brilliant green was found to be less toxic for tubercle bacilli in this medium than was malachite green. Although it is not as effective an antifungal agent as malachite green, the brilliant green, in combination with the Triton in the medium, seems to have an effective fungicidal result, since only 342 (1.5 per cent) of a total of 22,844 cultures were contaminated.[16]

This semi-synthetic medium was studied for its suitability for drug-susceptibility

testing on 50 strains of tubercle bacilli of various degrees of isoniazid resistance, as well as on 110 concentrated sputum specimens known to contain tubercle bacilli from isoniazid-treated patients. The results, when compared with those obtained on Peizer-Schechter and Löwenstein-Jensen egg media, showed that the semi-synthetic medium was suitable for direct and indirect drug-susceptibility tests of tubercle bacilli. However, further testing of the medium for this purpose in in progress.

The disadvantage of the synthetic medium, namely, the altered colony morphology of very young colonies of tubercle bacilli from the rough dry type to the smooth, is overweighed by its other assets. It is easy to prepare; the necessary ingredients are readily available; and the medium is reproducible and stable to heat on careful autoclaving. These advantages, along with its sensitivity for detecting viable tubercle bacilli from small inocula in processed specimens from isoniazid-treated patients, make this synthetic medium useful for routine diagnostic culturing of tubercle bacilli in a public health laboratory.

In summary: A new semi-synthetic agar medium for primary culturing of tubercle bacilli is described. It consists of the usual basic salts plus monosodium glutamate, potato starch, Triton WR 1339, sodium pyruvate, biotin, and Starlac®. Comparative tests made with 22,844 specimens from isoniazid-treated patients on the synthetic medium and on Löwenstein-Jensen and Peizer-Schechter media indicated that the synthetic medium was useful for routine diagnostic isolation of tubercle bacilli. It was found to be suitable for drug-susceptibility testing on 50 laboratory cultures of tubercle bacilli and on 110 concentrated sputum specimens known to contain tubercle bacilli.

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LACK OF CIRCULATING ANTIBODIES AFTER BCG IMMUNIZATION AS ASSAYED BY THE GLOBULIN TITRATION TECHNIQUE[1]

The demonstration of circulating antibodies in the serum of patients with active tuberculosis has been determined by a technique based on the theory that specific antibody globulin is extracted from a given serum by the antigen-antibody reaction with a formalinized polyvalent virulent *M. tuberculosis* antigen. The adsorbed antibody globulin was then assayed by a fall in titer of Coombs serum when human "O" positive erythrocytes sensitized with anti-D serum were used as the indicator system.[2, 3] The experiment describes the results obtained when BCG was administered to 17 normal healthy young adults.

A total of 95 medical students were tested prior to tuberculosis skin testing. None of these students demonstrated circulating antibody by the globulin titration technique. BCG was administered to 17 of these students who had negative skin test responses. Twenty-three days after immunization, no circulating antibody could be detected by the globulin titration technique in any of the 17 specimens of serum of the vaccinated students. Sera from 14 of these students were re-tested six to eight weeks after vaccination, with similar negative findings. The tuberculin skin test reaction in those tested after six weeks showed areas of 5 to 8 mm. of swelling and erythema. As has previously been reported,[2] the results with the globulin titration technique were independent of the tuberculin skin test.

Using the globulin titration technique, BCG, when administered to 17 human volunteers, produced no specific circulating antibody globulin comparable to that which has been demonstrated in active tuberculosis.

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[2] DOZIER, S. M. *et al.*: Am. Rev. Tuberc., 1957, *75*, 949.

[3] FUSILLO, M. H., *et al.*: Am. Rev. Tuberc., 1957, *75*, 954.

[4] The writers wish to acknowledge the efforts of Dr. L. W. Parr and the Department of Bacteriology as well as the Class of 1960, George Washington University School of Medicine, and Miss Carroll D. Possehl for their assistance in this study.

THE PANCREAS IN EXPERIMENTAL TUBERCULOSIS

Guinea Pig Inoculation via the Intraperitoneal Route

An early report by Opie[1] states that Carnot injected suspensions of *Myobacterium tuberculosis* directly into the pancreatic duct and into the parenchyma of the pancreas of the dog, but failed to induce characteristic tuberculous lesions. In human miliary tuberculosis, lesions arising in the pancreas are less frequently seen than those invading it by direct extension from an adjacent susceptible organ.[2] A review of the literature reveals no extensive reports on experimental tuberculosis of the pancreas in laboratory animals. During the examination of the spleens of guinea pigs inoculated intraperitoneally with *M. tuberculosis*, involvement of the pancreas was noted and further experiments were undertaken to determine the value of examining the pancreas routinely in the course of pathogenicity studies.

Two groups of guinea pigs were used. *Group A*, the controls, consisted of 12 OT-negative guinea pigs weighing from 300 to 800 gm. that provided normal organs for comparison. *Group B* consisted of 12 animals weighing from 395 to 925 gm. injected intraperitoneally with 1 mg. of virulent, culturally typical *M. tuberculosis* (No. 1047–54) isolated from a patient. *Group C* consisted of 24 guinea pigs weighing from 600 to 800 gm. which were inoculated intraperitoneally as in *Group B*. They were sacrificed for histopathologic study of the pancreas.

Group A: This group of normal animals was sacrificed and the weights of the spleen and pancreas were compared with those of the test groups. The median weight of the normal spleen was 0.68 gm. and that of the pancreas 1.73 gm. (figure 1).

Group B: The animals in *Group B* survived from eighteen to thirty-nine days. Five animals died at eighteen, 3 at twenty days; and 4 lived for more than thirty days. At necropsy the pancreas and spleen of each animal were removed and fixed in 10 per cent formalin, then weighed and sampled for tissue section. The median weight of the spleen was 2.38 gm. in tuberculous animals, and that of the pancreas was 7.31 gm.

Group C: The animals in *Group C* were destroyed at daily intervals, and tissue was examined to determine the extent of the disease in the pancreas.

Examples of normal and pathologic pancreases are shown as *A* and *B*, respectively, in figure 2.

Microscopic findings: Twenty-four hours after inoculation the acini of the secreting zymogen cells showed vacuolization and degeneration (figure 2*D*). Animals destroyed at forty-eight hours showed early tubercles forming in the loose connective tissue about the pancreas. Lymphangitis was common, the lymph vessels being distended with leukocytes; acid-fast bacilli were present in large clumps (figure 2*C*). Ghost acini were frequent at this stage of the infection. By the time the animal became moribund, the acini were usually completely lost. The time required varied with the strain and dosage, but in this experiment ghost acini predominated at fourteen to eighteen days. The islets remained intact longer than the cells of the acini but were finally replaced by granulomatous tissue. A normal guinea pig pancreas is shown in figure 2*E*.

In figure 3*A* ghost acini are shown within a spreading tuberculous process eighteen days

[1] Opie, E. L.: Disease of the Pancreas, J. B. Lippincott Co., Philadelphia, ed. 2, 1910, p. 237.

[2] Rich, A. R.: The Pathogenesis of Tuberculosis, Charles C Thomas, Springfield, Illinois, 1944, p. 317.

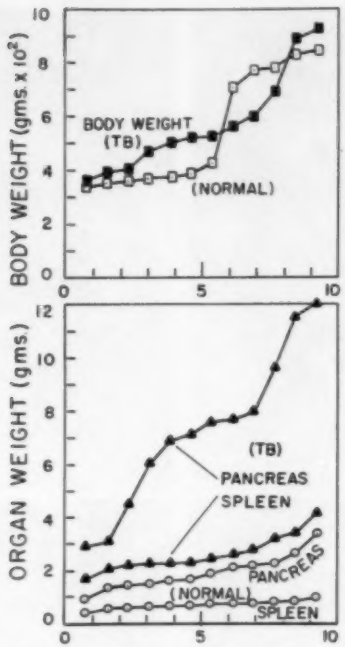

FIG. 1. Body and organ weights of normal and diseased animals.

after inoculation, at which time the animal was moribund. Acid-fast bacilli are shown in histiocytes at eighteen days (figure 3*B*).

As is well known, the spleen in guinea pig tuberculosis shows various degrees of involvement, often with only a few acid-fast bacilli demonstrable in the tubercles. In contrast, in nearly all cases the pancreatic tissue has been found in these studies to be completely destroyed, with little remaining recognizable architecture (figure 3*C*). Acid-fast bacilli are present in quantity as seen in figures 3*B* and 3*D*.

During the early phase of the infection (the second and third days), lymphangitis was observed in vessels in the loose connective tissue about the pancreas (figure 2*C*). Leukocytes, chiefly granulocytes and masses of acid-fast bacilli, distended the vessels. Following this phase the acini of the secreting zymogen cells began to degenerate and disappear, leaving only the acinar walls. Frozen sections stained with Sudan III showed the ghost acini to be filled with lipids. During this phase the islets remained intact, but might be isolated in a field of fat-filled acini. Tuberculous involvement of the pancreas appeared to take place by spreading along the interacinar spaces and finally extending into the acini (figures 3*A* and 3*C*).

The fact that subcutaneous and intramuscular inoculations do not lead to gross infection of the pancreas indicates that the organ is resistant to all except direct insults. Evidently the infection extends from the loose connective tissue at the site of inoculation by way of lymph vessels to the pancreas.

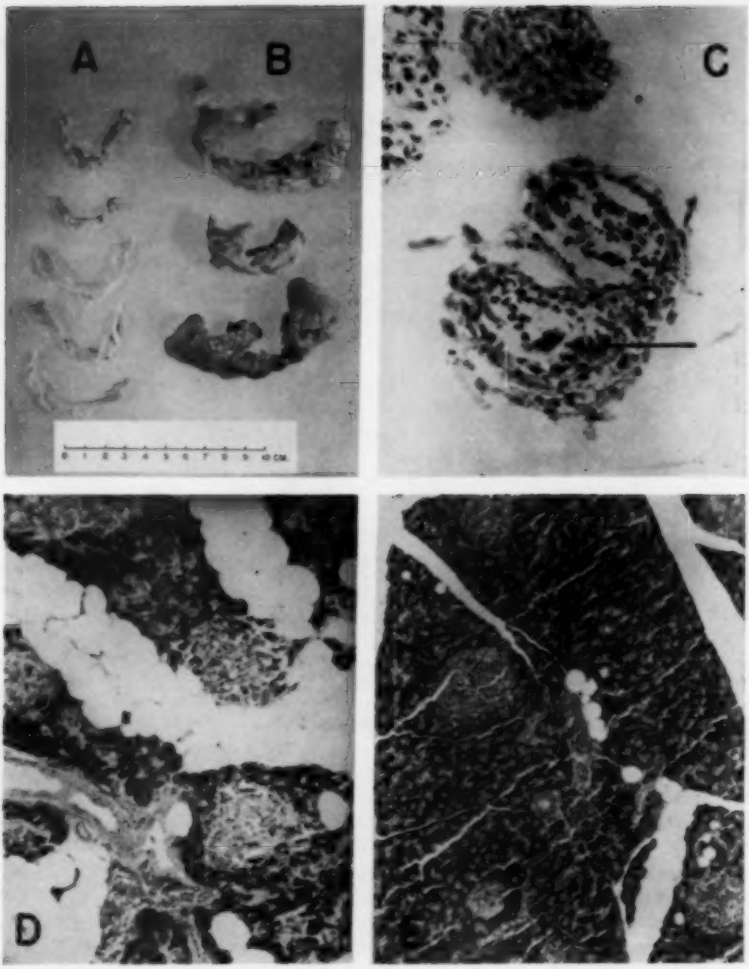

FIG. 2. *A*. Pancreas from uninoculated controls.

B. Pancreas from 3 animals inoculated eighteen days previously.

C. Pancreas forty-eight hours post infection. Peripancreatic lymphangitis with clusters of acid-fast bacilli (arrow). (Ziehl-Neelsen stain, × 350)

D. Acini of the zymogen secreting cells and islets twenty-four hours post infection showing early necrosis. (Ziehl-Neelsen stain, × 100)

E. Normal pancreas control group. (Ziehl-Neelsen stain, × 70)

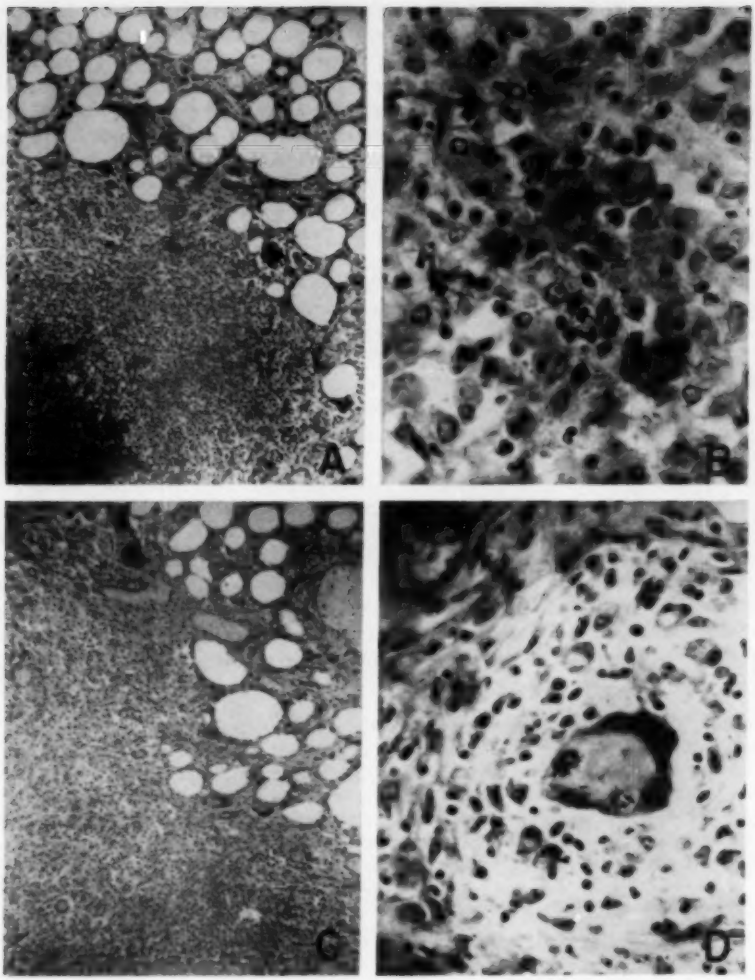

FIG. 3. *A*. Pancreas showing tuberculous pancreatitis eighteen days post infection. (× 75)
B. Pancreas eighteen days post infection showing acid-fast bacilli in histiocytes. (Ziehl-Neelsen stain, × 600)
C. Pancreas eighteen days post infection showing tuberculous inflammatory changes. (× 70)
D. Pancreas eighteen days post infection showing acid-fast bacilli in syncytial giant cells. (× 450)

In summary: Guinea pigs inoculated intraperitoneally with virulent *M. tuberculosis* developed extensive destructive tuberculosis of the pancreas. This is in marked contrast to the result in other routes of inoculation.

Tuberculous involvement of the pancreas appears to be due to the lymphatic spread and direct extension from tubercles forming in the afferent lymph capillaries and larger lymphatic vessels of the pancreas which drain the mesentery and omentum. The proportional increase in size of the pancreas and spleen is approximately equal.

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TITRATION OF CORD FORMATION AS A MEASURE OF PATHOGENICITY OF WILD-TYPE ACID-FAST BACILLI, TYPICAL AND ATYPICAL[1, 2]

A rapid test for the determination of virulence of tubercle bacilli has long been sought, but, to date, none has fully satisfied the requirements. In the course of work on cord formation of stock cultures of tubercle bacilli, it became clear that a particular culture appeared consistent in its capacity to form cords at a constant concentration of Tween® 80 in Dubos' Tween-albumin medium. This observation has led to a titration of cord formation of Mycobacteria[3] which gave stable and consistent end-points characteristically specific for each strain.

To further test the validity of these observations and, if possible, to give the test a fully practical turn, a more inclusive array of strains has been investigated. From numerous titrations of wild-type strains, 33 have been extensively studied for the interrelationships that might be associated with their pathogenicity. Twenty-one of these were typically rough strains of *M. tuberculosis* recovered from patients with pulmonary tuberculosis. Two were smooth strains, this being the only gross characteristic of differentiation. Ten were cultures of "atypical" strains isolated from patients who had no other known etiologic agent related to their pulmonary disease. The well-known stock "saprophyte," *M. phlei*, and the virulent H37Rv strain were studied concomitantly.[4] Colony morphology, susceptibility to isoniazid, catalase activity, cord titer, and Feldman's Index[5] of each have been determined.

The first group of "wild" strains were divided into: those that grew in the conventional incubation period of eight weeks or less, and those that required extended incubation for growth. The atypical strains had been previously classified as: photochromogens, skotochromogens, or Battey type, by Runyon,[6] who kindly provided them. All cultures were coded so that the identity of the strains was unknown to the investigators until the study was completed.

All cultures were maintained on Löwenstein-Jensen medium until three weeks prior to the study. Each culture was then transferred three times at eight-day intervals to liquid Tween-albumin medium to obtain dispersed growth. The cord titers were determined by the technique previously described.[3] Portions of the cultures used for cord-formation titrations were reserved for inoculation into guinea pigs. Two or more animals were inoculated with each culture and kept for a period of either five or eight weeks. The animals were sacrificed, and the extent of disease was noted and graded in keeping with the method described by Feldman, and was termed "Feldman's Index."

The susceptibility to isoniazid and the catalase activity of the cultures were also determined.

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[1] This study was made possible by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association, supplemented by assistance from the North Dakota Tuberculosis and Health Association, the North Carolina Tuberculosis Associations, and the Ruth Coltrane Cannon Research Fund.

[2] Presented in part before the Medical Session, as part of *Section 3B*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 20, 1958.

[3] MELVIN, I. G., and VANDIVIERE, H. M., Am. Rev. Tuberc., 1958, *78*, 83.

[4] The cord titer of *M. phlei* was 0.005, and that of H37Rv was repeatedly greater than 0.1.

[5] FELDMAN, W. H., Am. Rev. Tuberc., 1943, *48*, 248.

[6] Dr. Ernest Runyon, Veterans Administration Hospital, Sunmount, New York.

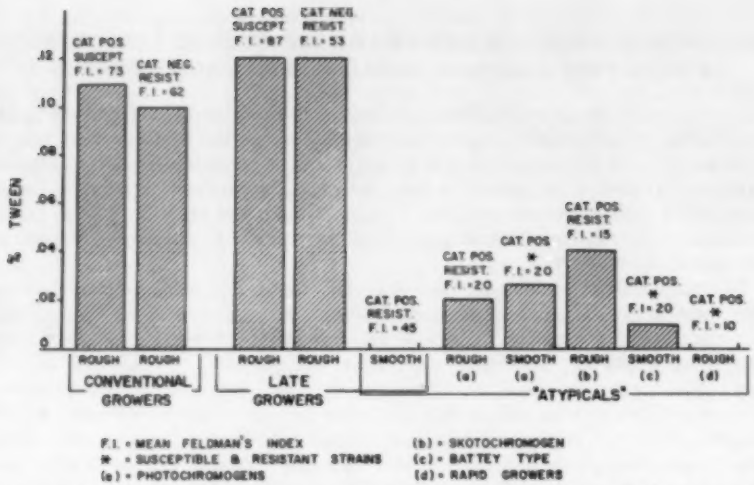

FIG. 1. The relationship between cord titration, isoniazid susceptibility, catalase activity, and virulence of "wild" strains ("typical" and "atypical").

The strains which were highly virulent for guinea pigs, producing a high Feldman's Index, also gave a high cord titer (more than 0.1). The only exceptions were the two smooth strains which did not form cords but produced a mean Feldman's Index of 45. It was noted in the previous study that smooth strains vary in capacity for cord formation and that cord titrations on smooth strains are not as reliable an index of virulence.

The atypical strains demonstrated only slight or no virulence for the guinea pigs, and also produced very low cord titers. Also, the cord titer of the rough, typical strains correlated well with the animal data.

The relationship for the various strains between cord titration, isoniazid susceptibility, catalase activity, and Feldman's Index is presented in figure 1. With isoniazid-resistant, catalase-negative strains, it was observed that the variation in cord titer was not significant and, although the Feldman Index was lowered, it was still significantly higher than that depicted for the atypical strains.

In general, it was found that the cord titers of rough wild strains were closely correlated with the degree of disease they produced when inoculated into guinea pigs. Therefore, it appears that the cord-formation titration is a reliable test for determining the degree of pathogenicity of wild rough strains of Mycobacteria.

* * *

It should be emphasized that, in order to obtain titers equivalent in value to those in the original titration established by this laboratory, the details of the technique used must be closely adhered to. The size of the culture containers and the volume and the depth of the medium (probably because of oxygen tension) play an extremely important part in determining the end-point of a titration. Varying these two factors can cause a two- or three-tube variation in titer.

The cord-formation titration has an advantage over animal inoculation as a test for virulence in that it can be completed in seven days with the dispersed suspension, whereas

inoculation of guinea pigs requires a longer period of observation and a greater expenditure of funds.

No change in titer is evident between the seven- and fourteen-day readings[3] except with the smooth bovine strains which did manifest a drop in titer.

In addition to its practical application to testing for virulence of routine cultures of Mycobacteria isolated from patients, the cord-formation titration has been incorporated by this laboratory as a regular procedure in potency standardization of antituberculosis vaccines. Also, it is used as a means of determining changes in virulence of stock laboratory strains of Mycobacteria, and has in every instance thus far correlated extremely well with tests for virulence in guinea pigs.

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June 19, 1958

BACTERIOTROPIC ACTIVITY OF ISONIAZID IN THE PRESENCE OF CERTAIN OTHER COMPOUNDS

As shown in a previous publication,[1] *p*-aminobenzoic acid (PABA) increases the activity of isoniazid against *M. tuberculosis in vitro*. A similar increase of isoniazid activity *in vivo*, if used together with PABA or some other arylamines, was described by other writers.[2, 3, 4] Therefore, it was considered of interest to investigate the influence of certain compounds (either structurally similar to PABA or known as metabolites involved in biochemical pathways of PABA) on the bacteriotropic potency of isoniazid *in vitro*.

The drugs tested were: PABA,[5] anesthesin,[6] procaine, tetracaine, folic acid (VCCHZ), isoniazid,[7] anthranilic acid, sulfanilic acid, DL-methionine, tryptophane, indole, uric acid,[8] and *p*-oxy-benzoic acid.[9] All of the drugs were sterilized for twenty minutes in flowing steam dissolved in either Long or Youmans liquid medium. Only isoniazid was dissolved in water and sterilized by Seitz filtration, and PABA was supplied in vials as a sterile aqueous solution of its sodium salt.

The simultaneous action of isoniazid and other drugs was at first tested on the *Mycobacterium* 607 strain. Youmans medium was distributed in 5 ml. amounts in tubes. These were then seeded with 0.1 ml. of a 10^{-1} mg. wet weight per ml. suspension of microorganisms in 0.85 per cent sodium chloride. Four parallel tubes were used for each drug or concentration. The results were read after seven days of incubation at 37°C. With the exception of isoniazid, none of the drugs used was found to be bacteriostatic by itself in the tested concentration.

As shown in table 1, PABA, tryptophan, and indole increased the activity of isoniazid *in vitro* against *M.* 607. Uric acid, on the contrary, decreased the isoniazid inhibition of this strain. All of the other drugs tested were ineffective in both ways. Thus, at least for this strain, it may be concluded that either a free carboxyl group or a free amino group of PABA is essential to increase the activity of isoniazid *in vitro*. The "para" configuration of both radicals seems to be of similar importance.

In order to ascertain the obtained results, another set of experiments was started with the H37Rv strain. Long medium was used in this study, and a description of the method may be found elsewhere.[1]

That PABA, tryptophan, and indole increase the activity of isoniazid against the H37Rv strain *in vitro* may be seen in table 2. In this case, uric acid was ineffective in either way. It is of interest that the bacterial mass growing in the presence of indole, as well as the medium, is red-brown pigmented. This pigmentation is not dependent upon the presence of isoniazid, but it is slightly more pronounced if the latter drug is added to the medium. Similar pigmentation was observed by Mayer[10] in a strain of *M.* 607 when grown in the presence of PABA.

[1] URBANČÍK, R.: Am. Rev. Tuberc., 1957, *76*, 706.
[2] BRETTONI, B.: Minerva med., 1952, *2*, 674.
[3] JOHNSON, W., AND CORTE, G.: Proc. Soc. Exper. Biol. & Med., 1956, *92*, 446.
[4] BELL, J. C., and MITCHELL, R. S.: Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration—Armed Forces, 1957, p. 105.
[5] Kindly supplied as Paraminan® by R. Gallier, Paris.
[6] Kindly supplied by E. Ritsert, Frankfurt.
[7] The four preceding drugs were obtained from Spofa, Czechoslovakia.
[8] The six preceding drugs were obtained from Merck, Germany.
[9] Obtained from Wild, Germany.
[10] MAYER, R. L.: J. Bact., 1944, *48*, 337.

TABLE 1

THE INFLUENCE OF CERTAIN DRUGS ON THE ACTIVITY OF ISONIAZID AGAINST MYCOBACTERIUM 607

Drug	Concentration in γ per ml.	Plus γ per ml. of Isoniazid		
		5	10	25
0	—	+	+	0
PABA	200	+	0	0
Anaesthesine	50	+	+	0
Procaine	200	+	+	0
Tetracaine	100	+	+	0
Folic acid	200	+	+	0
Anthranilic acid	100	+	+	0
Sulfanilic acid	50	+	+	0
p-Oxy-benzoic acid	200	+	+	0
Sulfanilamide	50	+	+	0
Methionine	50	+	+	0
Tryptophan	100	+	0	0
Indole	50	(+)	0	0
Uric acid	100	+	+	+

\+ = both submerged and surface growth
(+) = only submerged growth
0 = no growth

TABLE 2

THE INFLUENCE OF CERTAIN DRUGS ON THE ACTIVITY OF ISONIAZID AGAINST THE H37Rv STRAIN

Drug	Concentration in γ per ml.	X	T-Test (95 Per Cent Confidence Limits)
0	0	0.3121	(0.2875 — 0.3367)
Isoniazid	0.05	0.0591	(0.0484 — 0.0698)
Isoniazid	0.05	0.0421	
Plus PABA	+200		
Isoniazid	0.05	0.0235	
Plus indole	+50		
Isoniazid	0.05	0.0412	
Plus tryptophan	+100		
Isoniazid	0.05	0.0598	
Plus uric acid	+100		
Isoniazid	0.05	0.0215	
Plus PABA	+200		
Plus indole	+50		
Indole	50	0.2983	
Uric acid	100	0.3355	

X = dry weight of the bacterial mass, mean value in grams from three to four parallel flasks

TABLE 3

THE INFLUENCE OF CERTAIN DRUGS ON THE ACTIVITY OF ISONIAZID AGAINST THE ATYPICAL PHOTOCHROMOGEN 4

Drug	Concentration in γ per ml.	X	T-test (95 Per Cent Confidence Limits)
0	0	0.2968	(0.2811 – 0.3125)
Isoniazid	1	0.0767	(0.0629 – 0.0805)
Isoniazid	1	0.0533	
Plus PABA	+200		
Isoniazid	1	0.0285	
Plus indole	+50		
Isoniazid	1	0.0368	
Plus tryptophan	+100		
Isoniazid	1	0.0698	
Plus uric acid	+100		
Isoniazid	1	0.0133	
Plus PABA	+200		
Plus indole	+50		

X = dry weight of bacterial mass, mean value in grams from three to four parallel flasks

Using the same medium and method, a third experiment was performed with the atypical photochromogen 4.

The results given in table 3 correspond with those obtained with the H37Rv strain. However, only slightly more pronounced pigmentation was observed in the bacterial mass grown on the medium with indole, compared with the color of the colonies in the control flask.

The explanation of the facts presented is not simple. It is of interest that sulfanilamide, a drug reported to decrease[9] the acetylation of isoniazid *in vivo*, had no effect under the conditions of the present experiments. This finding does not correspond with the results of similar experiments presented by Gillissen.[11]

It was recently suggested by Krüger-Thiemer[12] that isoniazid activity might depend on the competitive inhibition of enzymatic complexes by isonicotinic acid, using nicotinic acid or its derivatives. Moreover, there are now certain suggestions indicating a metabolic relationship between nicotinic acid and tryptophan or indole. Marnay[13] was of the opinion that, in *E. coli*, nicotinic acid might be synthetized from tryptophan. Sevage and Green[14] reported that PABA was derived from tryptophan by a staphylococcal strain.

Thus, it may be said that either PABA, tryptophan, or indole are somehow connected with the synthesis of nicotinic acid. However, little is known about these pathways in the case of Mycobacteria. Therefore, many further experiments are needed to give a more precise explanation of the facts here presented.

In summary: PABA, tryptophan, and indole increased the activity of isoniazid against three different strains of Mycobacteria *in vitro*. The activity of PABA seems to depend upon its chemical configuration. Uric acid decreased the activity of isoniazid in the case

[11] GILLISSEN, G.: Ztschr. Hyg., 1953, *138*, 321.
[12] KRUGER-THIEMER, E.: Jber. TuberkForsch. Borstel, 1956, *57*, 301.
[13] MARNAY, C.: Bull. Soc. chim. biol., 1951, *33*, 174.
[14] SEVAGE, M. G., and GREEN, M.: J. Bact., 1944, *47*, 450.

of *M.* 607, but not in the cases of "typical" and "atypical" virulent strains of Mycobacteria.

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METABOLISM OF ISONIAZID BY MYCOBACTERIUM TUBERCULOSIS BCG WITH REFERENCE TO CURRENT THEORIES OF THE MODE OF ACTION

A theory of the mode of action of isoniazid, recently described in this journal,[1] was based on the supposition that organisms susceptible to isoniazid do not take up *iso*nicotinic acid and that, when *iso*nicotinic acid is formed in the cells, it accumulates and disturbs the normal metabolism of the cells by competing with nicotinic acid. Some work presently being undertaken in these laboratories is highly relevant to the above theory. Some of the results have already been submitted for publication,[2] and others will be described more fully later.

The following experiments have been made with a strain of BCG susceptible to 0.06 γ of isoniazid per ml. and a strain resistant to 40 γ per ml. derived from the wild type.[2]

The organisms were grown on the surface of Sauton medium, harvested and worked to a paste with a spatula on filter paper, then suspended in phosphate buffer pH 7.

Radioactive *iso*nicotinic acid was prepared from radioactive isoniazid,[3] labeled in the carbonyl group, by oxidation with bromine and the product was tested chemically for *iso*nicotinic acid and isoniazid.

Chemical estimations were made on the supernatants from suspensions exposed to isoniazid. For the estimation of isoniazid the color produced with picryl chloride under alkaline conditions was used[4]; and, for *iso*nicotinic acid, the color produced with cyanogen bromide and buffered ammonia reagent[5] was used.

No color developed with cyanogen bromide and "metol." This test is positive with nicotinic acid but not with *iso*nicotinic acid[6] and, therefore, eliminated the possibility that the color measured in the cell supernatants was due to nicotinic acid. None of these reagents gave color with cell supernatants in the absence of added isoniazid.

In figure 1 the metabolism of isoniazid by susceptible BCG is shown. All of the data are expressed as γ of isoniazid per ml. so that results are directly comparable. The figure shows the residual isoniazid, the *iso*nicotinic acid formed, and the concentration bound in the cells. The cell content was estimated from the radioactive count per milligram dry weight and the known cell dry weight of the suspension. It may be seen that there is also a breakdown of isoniazid in the presence of heat-killed cells, although at a slower rate.

These results are in agreement with Krüger-Thiemer's suggestion[1] that *iso*nicotinic acid is formed in the cells. It should be noted, however, that the *iso*nicotinic acid is found in the supernatants in concentrations which approximate closely the theoretic yields from the isoniazid lost and that there is no apparent lag between the disappearance of isoniazid and the appearance of *iso*nicotinic acid. This shows that *iso*nicotinic acid leaves the cell readily and is not in agreement with the theory that the action of isoniazid involves the accumulation of *iso*nicotinic acid in the cell. Measurement of the uptake of radioactive *iso*nicotinic acid showed that the acid also enters the cells rapidly.

The uptake of isoniazid and of *iso*nicotinic acid in the presence of susceptible and

[1] KRÜGER-THIEMER, E.: Am. Rev. Tuberc., 1958, 77, 364.
[2] YOUATT, J.: Australian J. Exper. Biol. and M. Sc., 1958, (in press).
[3] Obtained from the Radiochemical Centre, Amersham, England.
[4] CUTHBERTSON, W. F. J., AND IRELAND, D. M.: Biochem. J., 1952, *52*, XXXIV.
[5] RUBIN, S. H., *et al.*: Dis. Chest, 1952, *21*, 439.
[6] WOJAHN, H.: Jber. TuberkForsch. Borstel, 1952-1953, p. 726.

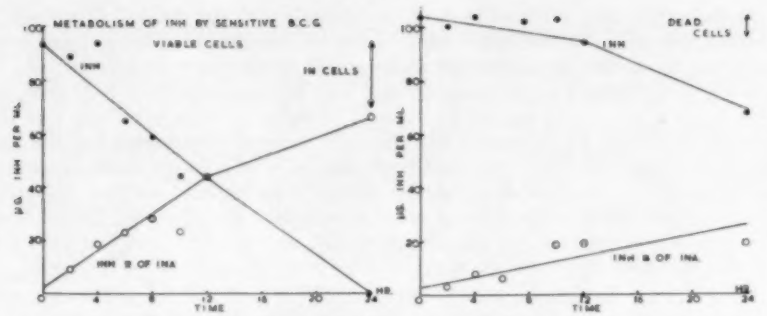

FIG. 1. The metabolism of isoniazid by susceptible BCG.

TABLE 1

UPTAKE OF ISONIAZID AND ISONICOTINIC ACID BY BCG

	Radioactivity Counts	
	Isoniazid	*Iso*nicotinic Acid
	min/mg.	
Viable isoniazid-susceptible BCG	106	160
Viable isoniazid-resistant BCG	45	193
Killed susceptible and resistant BCG	50	15

Washed cells suspended in phosphate buffer were shaken in air with 100 γ of isoniazid per ml. or with the equivalent amount of *iso*nicotinic acid. Dead cells were killed by heat at 245°F. Samples were washed four times rapidly before drying and counting. The results are expressed as counts per mg. dry weight of organisms to permit direct comparison. Time of incubation, two hours at 37°C.

resistant BCG may be seen in table 1. Viable and resistant organisms were found to take up little isoniazid over periods of several hours even with concentrations considerably in excess of the resistance level of the cells as measured by growth.[2]

Suspensions were shaken in air with radioactive isoniazid at 100 γ per ml. and with the equivalent concentration of radioactive *iso*nicotinic acid at 37°C. for two hours. The results are expressed as counts per minute per milligram dry weight and are, therefore, directly comparable. The reason for the discrepancy between these results and those of Barclay and associates,[7] who found that *iso*nicotinic acid was not taken up by cultures, is not known.

Krüger-Thiemer[1] postulated that the interference with dehydrogenase-cytochrome systems resulted in an increased activity of flavine enzymes and consequent increase in hydrogen peroxide formation. It is not clear whether the theory postulates that the rate of production of peroxide becomes greater than the rate of removal by catalase and peroxidase or whether these enzymes are also considered to be inhibited. Holmes and Rubbo[8] have suggested from different evidence that the accumulation of peroxide is an unlikely mechanism of isoniazid action.

[7] BARCLAY, W. R., *et al.*: Am. Rev. Tubere., 1954, *70*, 784.

[8] HOLMES, I., AND RUBBO, S. D.: Nature, 1958, *181*, 1203.

Nicotinic, picolinic, and benzoic acid hydrazides inhibit the uptake of radioactive isoniazid and are themselves metabolized by the cell suspensions of BCG. These compounds are considerably less inhibitory to the growth of Mycobacteria than isoniazid.

It has been observed by the present writer that isoniazid-susceptible BCG cells develop a yellow color when exposed to isoniazid in buffer solutions pH 7 and that the pigment diffuses into the suspending fluid. In order to assess the importance of this pigment production, it has been considered in the light of what is known about the uptake and breakdown of isoniazid. Reference has already been made to the fact that some uptake and breakdown occurs in the presence of dead cells. For simplicity, this low level of activity will be regarded as a negative result and, when a positive result is quoted, it will refer to uptake or metabolism in excess of the levels characteristic of dead cells.

The provision of adequate aeration was essential for the uptake, breakdown, and pigment production by drug-susceptible cell suspensions. The endogenous respiration rate of Mycobacteria is very high. As a result, the density of the suspension became limiting for cells shaken in air. Anaerobic suspensions showed no uptake, breakdown, or pigment production; and aerobic, stationary suspensions behaved similarly, indicating that the dissolved oxygen was negligible within a few minutes of standing.

Although the breakdown of isoniazid to *iso*nicotinic acid has been observed with resistant BCG, the uptake of radioactive isoniazid is no greater than that of killed cells, and pigment production has not been observed.

The processes of uptake, breakdown, and pigment production by suspensions in phosphate buffer pH 7 are all inhibited by NaCN and NaN_3 at M/1000 and are not affected by the addition of iron salts or chelators such as versene or *o*-phenanthroline. The latter compound stained the cells pink and the color was not removed by washing.

The pigment production appeared to be specifically related to isoniazid since it was not observed with any of the following compounds: streptomycin, 100 γ per ml.; para-aminosalicylic acid (PAS), 1,000 γ per ml.; thiosemicarbazone, 100 γ per ml.; pyrazinamide, 100 γ per ml.; hydrazine hydrochloride, 100 γ per ml.; hydrogen peroxide, 100 γ per ml.; benzoic, nicotinic, or picolinic acid hydrazide, 100 γ per ml.; versene, M/1,000; *o*-phenanthroline, M/1,000; *iso*nicotinic acid, 100 γ per ml.

A yellow compound was produced in the presence of PAS, but this differed from the isoniazid-stimulated pigment by its insolubility in acid, its solubility in ethyl acetate, and its absorption maximum at 425μ.

It is too early to say much of the nature and significance of the yellow pigment. Paper chromatography has shown that there are several components but, as the colored material is not stable, it is not known whether they are all derived initially from one compound. Direct measurement of the spectrum of cell supernatants after exposure to isoniazid shows one peak only between 300 and 700μ. It was not possible to measure in the ultraviolet region because of the presence of residual isoniazid and *iso*nicotinic acid. The peak in absorption occurred at 450–460μ. In alkaline solution the color was lost, and in acid solution it was intensified. Yellow degradation products were obtained by boiling in alkaline solution and in the presence of dithionite. These no longer showed a peak absorption at 450μ. The color in the cell supernatants was photolabile. The color could be extracted from aqueous solution into molten phenol or butanol but not into ether, ethyl acetate, benzene, or chloroform. The pigments show a yellow fluorescence under ultraviolet light, although some of the minor bands which may be degradation products show no fluorescence or blue fluorescence. Similarities exist between the characteristics of these compounds and those of flavines which make it tempting to suggest that they

are the result of a disordered flavine synthesis, but there are not sufficient data to warrant this conclusion.

At present it is easier to discard theories of the mode of action of isoniazid than to replace them. No evidence has been obtained by the writer which favors the theory that isoniazid acts by virtue of its chelating ability[9] or that it inhibits the transaminase systems.[10] The results described here also fail to support the hypothesis that isoniazid toxicity is due to its breakdown to *iso*nicotinic acid.[1] The nitrogenous products of isoniazid breakdown have not been identified, but there is difficulty in assigning an inhibitory role to a nitrogenous product since the relatively inactive nicotinic acid hydrazide is also metabolized and may be expected to give similar nitrogenous products. The close relationship which exists between the appearance of yellow pigments and the metabolism of isoniazid by isoniazid-susceptible organisms gives rise to the hope that these phenomena may supply a clue to the mode of action of the drug.

School of Bacteriology, University of Melbourne
Carlton, N. 3, Australia

Jean Youatt

July 24, 1958

[9] Cymerman-Craig, J., *et al.*: Nature, 1955, *176*, 34.
[10] Youatt, J.: Biochem. J., 1958, *68*, 193.

Correspondence

INTERNATIONAL UNION AGAINST TUBERCULOSIS

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

This letter is with regard to an important omission in my article on the International Union Against Tuberculosis published in the January issue. In that article my review of the accomplishments of the Union in recent years was necessarily quite incomplete, and one of several omissions was my failure to state that simultaneous interpretation was first provided at the Union's 1954 Conference in Madrid under the chairmanship of Dr. Jose Alix y Alix and during the presidency of Professor Crespo Alvarez. This was indeed a milestone in the history of the progress of the Union and should have been mentioned specifically in my article.

At the recent interim business session of the Union in Paris in July a number of delegates spoke to me about my article. These comments were favorable for the most part, but it was called to my attention that the article gave the false impression that the 1957 New Delhi conference was the first one at which simultaneous interpretation had been provided.

It is true that at New Delhi panel discussions and seminars were attempted for the first time, sessions being conducted simultaneously. These were possible because of the unusually complete and extensive built-in simultaneous interpretation facilities in the new building constructed in New Delhi in 1956 specifically for international congresses. But this in no way detracts from the history-making significance of the initial provision for simultaneous interpretation in the main hall in which essentially all of the sessions were held at the splendid Madrid conference. Those of us who are not expertly multilingual were enabled at Madrid for the first time to get the full significance of each presentation at the time of delivery at an international tuberculosis conference sponsored by the International Union Against Tuberculosis. The country of Spain, and particularly Dr. Alix y Alix and Professor Crespo Alvarez, deserve full credit for this momentous step forward, as well as for one of the finest scientific and warmly hospitable international conferences ever held.

James E. Perkins
Managing Director
National Tuberculosis Association
of the United States of America

August 13, 1958

Books

THE BACTERIOLOGY OF TUBERCULOSIS. By Egon Darzins. Pp. 488, University of Minnesota Press, Minneapolis, Minnesota, 1958. Price, $10.00.

Dr. Darzins' prodigious review of tuberculosis research has as one stated purpose the suggestion of new problems for study by tracing the background of current and past problems. Quite aside from the success or failure of his book in this regard, Dr. Darzins has nonetheless compiled a huge body of knowledge and research and classified it into five principal parts: morphology and cytology, energy sources and growth, isolation and identification, types and pathogenicity, and experimental tuberculosis.

By and large, each of the thirty-three chapters is a fairly complete review dealing with a single topic without much continuity with the other chapters except for citations which are repeated when applicable.

The example that comes first to mind concerns the somewhat arbitrary separation into three chapters of Part One, the so-called physical, biologic, and chemical methods for identifying cell structures. Thus, staining with a fluorescent dye is cited as a physical method, staining for substances broken down or not broken down by acid or enzymes as a biologic method, while staining for nucleic acids and other known substances is considered a chemical method. Actually there is considerable exchange of material among these three chapters. To this reviewer the better solution would have been to group all staining procedures under one heading, for, taken all together, the collected information comprises an excellent review of cytochemical techniques available for the study of microorganisms in general and mycobacteria in particular.

A gratifying feature of Dr. Darzins' work is that a large proportion of his fifty-page bibliography is devoted to establishing a sturdy biochemical, biologic, and microbiologic framework in which to place tuberculosis research. In his chapters in Part Two on sources of energy and growth, after a brief orientational and historic introduction describing the fundamentals of oxidation, fermentation, and growth throughout the living world, he turns to mycobacteria in particular. When parts of the over-all picture are missing for the tubercle bacilli or its close relatives, the blanks are filled in by references to pertinent experiments with other organisms.

Another purpose for preparing this book, we are told in the preface, is to aid workers in the field in avoiding energy-wasting repetition of experiments already done by others. Only after examination of the five-page tabulation of results obtained by different investigators concerned with the effect on growth and respiration by various possible carbon sources can this purpose be viewed in its proper perspective. A glance will demonstrate to the reader that, with the exception of oleic acid, streptomycin, and isoniazid in a list of more than forty compounds, the effects of a substance on the growth of tubercle bacilli are as varied and numerous as there are investigators to perform experiments. The fifth section, dealing with problems of experimentation (and perhaps the most valuable to research workers), discusses among other things statistical traps, inherent and human error in bacteriologic work, pitfalls of drug testing, and physiologic and pathologic variation among populations of human subjects and laboratory animals. Indeed, there is little if anything reported in the entire four hundred pages to discourage one from repeating experiments already reported by others.

Respect for history and anteriority is a laudable quality for those writers reviewing the

literature for a period of nearly seventy-five years, but the virtues of a book of this type are often not apparent until subjected to use by those most concerned with its subject matter. Faults, such as major errors and omissions, on the other hand, may be immediately apparent. Of the former, one could mention but few indeed save perhaps of judgment in the selection of the photographic illustrations. These, with few exceptions, are poor. As for omissions, one might have have hoped for a chapter surveying the immunology of tuberculosis. What is missed most, however, is a critical statement now and then. The serious reader and certainly the beginner will want to supplement this reading with more critical reviews.

The Bacteriology of Tuberculosis is a good reference book for a medical library or a microbiologic laboratory, and Dr. Darzins is certainly to be applauded for compressing so much information into so convenient a package.

CURTIS A. WILLIAMS, JR.
New York, New York

ROENTGENOLOGY OF THE CHEST. Edited by Coleman B. Rabin. Pp. 484, Charles C Thomas, Springfield, Illinois, 1958. Price, $19.50.

The purpose of this book, as stated in the preface, is "to present roentgenology of the chest to the roentgenologist from the clinical standpoint, and to the clinician from the radiological point of view." This volume fulfills the above intentions admirably.

It is a book of 484 pages with excellent paper and very readable typography, and the illustrations are beautifully reproduced. Each chapter contains a large bibliography which is up to date, and each one of the contributors seems well qualified to author the section assigned. The index is complete, and the entries are cross-indexed.

The first and second chapters are well conceived. The first, by Rigler, on the possibilities and limitations of roentgenographic diagnosis gives a broad view of the value of the roentgenographic examination. The second, "Normal Roentgen Anatomy," by J. P. Medelman is concise yet thorough, and presents the basic anatomy necessary for evaluation of the chest and its contents. Both seem excellent choices as fundamental chapters for the specialist in thoracic diseases. The chapters on tuberculosis, the fungal diseases, and the pneumonias, on the other hand, are written by clinicians with adequate emphasis placed on clinical findings and correlation with the roentgenogram. The use of the classification of "putrid" and "nonputrid" pulmonary abscesses is established clinically, but is little used in roentgenographic classification. Likewise, one might object to the subjective descriptions of "hard" and "soft" shadows mentioned in the chapters on tuberculosis; but widespread use may justify these phrases.

Several chapters are dedicated to technical and physiologic aspects of roentgenography of the chest. The chapters on the bronchogram, sectional radiography, kymography, and angiocardiopneumography are both interesting and illustrative. The chapters pertaining to the heart and great vessels are generally well written and are profusely illustrated with routine films and angiocardiograms, as well as explanatory line drawings.

Especially worth while is the chapter by Felson on special signs in chest roentgenology. He points out the value of the silhouette sign, the "S" line of Golden, the sail sign, and many others, describing each in detail and explaining its significance. These diagnostic aids are widely used by the roentgenologist.

Some of the principles of mass chest fluorography outlined here do not have wide acceptance, and the use of positive reproductions in Kerly's chapter on sarcoidosis is unfortunate. The criteria given for exploration versus observation of the parenchymal

nodule are also open to criticism. However, these are minor criticisms of an otherwise excellent volume, which can be wholeheartedly recommended to both clinicians interested in chest diseases and roentgenologists.

FREDERICK W. DECK
New York, New York

BASIC FACTS OF MEDICAL MICROBIOLOGY. By Stewart M. Brooks. Pp. 306, W. B. Saunders Company, Philadelphia, Pennsylvania, 1958. Price, $4.75.

This book is designed "to present the basic facts of medical microbiology to those preparing for the professions which require an intelligent understanding of the subject." This aim is achieved in a didactic manner, and a remarkable compilation of facts has been compressed into a small space. The whole sphere of microbiology is encompassed, including infestations and even the clinical aspects of venomous bites. A useful appendix and glossary for beginners are added and altogether the book is concise and for the most part clearly written, and there are profuse illustrations of a moderate standard.

The compression of such a vast amount of knowledge is bound to bring about a certain lack of balance and relative accentuation of less important matter. In a small section devoted to serum hepatitis, although the use of the same syringe for more than one patient is deprecated, no mention is made of the needle or the importance of effective sterilization of both after their use. Inaccuracies and ambiguities are, however, very few, and this book can be recommended for students in the ancillary branches of the profession, particularly nurses and laboratory technicians.

JOHN BATTEN
London, England

BOOKS RECEIVED

REHABILITATION AFTER ILLNESS AND ACCIDENT. Edited by Thomas M. Ling and C. J. S. O'Malley. Pp. 119, The Williams & Wilkins Co., Baltimore, 1958. Price, $3.50.

THE PSYCHOLOGY OF MEDICAL PRACTICE. By Marc H. Hollender. Pp. 276, W. B. Saunders Company, Philadelphia, 1958. Price, $6.50.

DIE INFEKTIONSKRANKHEITEN DES MENSCHEN UND IHRE ERREGER, Vols. I and II. By A. Grumbach and W. Kikuth. Pp. 1,702, Georg Thieme Verlag, Stuttgart, 1958. Price, $47.15.

RONTGENANATOMIE DER ENUGEBORENEN- UND SAUGLINGSLUNGE. By Zoltan Zsebök and R. Glauner. Pp. 160, Georg Thieme Verlag, Stuttgart, 1958. Price, $17.85.

ÜBER DEN EINFLUSS VON PHYSIKALISCHEN UND CHEMISCHEN FAKTOREN AUF DIE CYTOLOGIE DER TUBERKELBAZILLEN UND ANDERER MYCOBAKTERIEN. By Werner Roth. Pp. 77, Verlag Helbing & Lichtenhahn, Basel, 1958. Price, SFr. 11.50.

LE PNEUMOTHORAX SPONTANÉ NON TUBERCULEUX DE L'ADULTE ET SON TRAITEMENT. By André Meyer, J. P. Nico, and J. Carraud. Pp. 144, Masson & Cie, Paris, 1958. Price, 1,950 fr.

LE MÉDIASTIN ET SA PATHOLOGIE. By Maurice Bariéty and Charles Coury, Pp. 854, Masson et Cie, Paris, 1958. Price, 11,000 fr.

TRATAMIENTO Y PROFILAXIS DE LA TUBERCULOSIS. By Luis Sayé. Pp. 541, Salvat Editores, S. A., Barcelona, 1958.

American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1959—Chicago, Illinois—May 24–29
1960—Los Angeles, California—May 15–20

MEDAL AWARDS

The chairmen of the Trudeau and Ross Medal Committees are requesting suggestions for possible recipients of the respective NTA medals to be awarded at the annual meeting in Chicago in May, 1959.

The Trudeau Medal is awarded annually for "the most meritorious contributions on the cause, prevention, or treatment of tuberculosis."

The Ross Medal is awarded for "outstanding contributions to the tuberculosis control movement." Specific criteria governing the selection of candidates for the Ross Medal are available upon request from the NTA.

Names with supporting data should be sent directly to the respective chairmen: *Trudeau Medal*—William L. Cooke, M.D., 230 Professional Building, Charleston, West Virginia; *Ross Medal*—Peter W. Janss, Suite 212, Equitable Building, Des Moines 9, Iowa.

Suggestions should be received by February 1, 1959.

1959 PRIZE ESSAY CONTEST

The American College of Chest Physicians is offering three cash awards to winners of the 1959 prize essay contest. First prize, $500; second prize, $300; third prize, $200. Each winner will also receive a certificate.

The contest is open to *undergraduate* medical students throughout the world. Essays may be written on any phase of the diagnosis and treatment of chest diseases (cardiovascular or pulmonary). The contest closes on April 15, 1959. For application and further information, write to: American College of Chest Physicians, 112 East Chestnut Street, Chicago 11, Illinois, U. S. A.

SECOND INTERNATIONAL CONGRESS OF INFECTIOUS PATHOLOGY

The Second International Congress of Infectious Pathology will be held in Milan, Italy, May 6–10, 1959. All communications concerning the Congress should be addressed to Professor Carlo Zanussi, General Secretary, Via Francesco Sforza, Milano, Italy.

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1. Alexander, L.: Chemotherapy of depression—Use of meprobamate combined with benactyzine (2-diethylaminoethyl benzilate) hydrochloride. J.A.M.A. 166:1019, March 1, 1958. 2. Current personal communications; in the files of Wallace Laboratories.

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NATIONAL TUBERCULOSIS ASSOCIATION 1904–1954

A Study of the Voluntary Health Movement in the United States

by Richard Harrison Shyrock, Ph.D.

W. H. Welch Professor of the History of Medicine, and Director of the Institute of the History of Medicine, The Johns Hopkins University.

348 pages, illustrated $5.50

This book would be a valuable addition to any medical library. It should be on the reference list of every medical school; and on the desk of all private physicians interested in the problems of tuberculosis.

Not only does it supply a historical background for the antituberculosis movement in the United States, but it affords enlightening orientation to the entire voluntary health movement with the National Tuberculosis Association as the principal illustration.

The three overlapping areas of the Association's activities—medical science and practice, public health, and social welfare—are presented here by an able medical historian who knows how to give facts in a clear and interesting way.

Among the chapters are: Medical Background; Social Background; Tuberculosis in the United States; 1904; Founding of the National Tuberculosis Association; Arousing the Public, 1904–1908; Growth of the Tuberculosis Movement, 1904–1920; Administrative Developments and Reorganization; Problems and Policies, 1908–1922; Widening Contacts and Activities, 1917–1922; Foreign Contacts, War, and the Emergence of a New Era; The Research Program, 1940–1954; Chemotherapy and the Climax of Fifty Years of Service.

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